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EXHIBIT A

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A Randomized, Double-Blind, Study of Rofecoxib in Patients with Mild Cognitive Impairment

Leon J Thal[1], Steven H Ferris[2], Louis Kirby[3], Gilbert A Block[4], Christopher R Lines*[,4], Eric Yuen[4], Christopher Assaid[4], Michael L Nessly[4], Barbara A Norman[4], Christine C Baranak[4] and Scott A Reines[4], on behalf of the Rofecoxib Protocol 078 study group[5]

[1]University of California, San Diego, CA, USA; [2]New York University School of Medicine, New York, NY, USA; [3]Pivotal Research Centers, Peoria, AZ, USA; [4]Merck Research Laboratories, West Point, PA, USA

Inflammatory mechanisms have been implicated in Alzheimer's disease (AD) and might be mediated via the COX-2 enzyme. Previous studies with the selective COX-2 inhibitors, rofecoxib and celecoxib, have shown that they do not alter the progression of AD. We conducted a double-blind study to investigate whether rofecoxib could delay a diagnosis of AD in patients with mild cognitive impairment (MCI), a group with an expected annual AD diagnosis rate of 10–15%. MCI patients ⩾65 years were randomized to rofecoxib 25 mg ($N=725$) or placebo ($N=732$) daily for up to 4 years. The primary end point was the percentage of patients with a clinical diagnosis of AD. The estimated annual AD diagnosis rate was lower than the anticipated 10–15%: 6.4% in the rofecoxib group *vs* 4.5% in the placebo group (rofecoxib : placebo hazard ratio = 1.46 (95% CI: 1.09, 1.94), $p=0.011$). Analyses of secondary end points, including measures of cognition (eg the cognitive subscale of the AD Assessment Scale (ADAS-Cog)) and global function (eg the Clinical Dementia Rating (CDR)), did not demonstrate differences between treatment groups. There was also no consistent evidence that rofecoxib differed from placebo in *post hoc* analyses comparing ADAS-Cog and CDR-sum of boxes scores in overlapping subgroups of patients who had Mini Mental State Exam scores of 24–26 in the present MCI study and in a previous AD treatment study with a similar design. The results from this MCI study did not support the hypothesis that rofecoxib would delay a diagnosis of AD. In conjunction with the lack of effects observed in previous AD studies, the findings suggest that inhibition of COX-2 is not a useful therapeutic approach in AD.

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INTRODUCTION

Neuropathological and epidemiological data suggest that inflammatory mediators and immune mechanisms may play a role in the pathogenesis of Alzheimer's disease (AD) (McGeer *et al*, 1996; Cagnin *et al*, 2001; Ho *et al*, 2001; in 't Veld *et al*, 2002; Etminan *et al*, 2003). It has been proposed that these effects may be mediated via the COX-2 enzyme (Pasinetti, 2001). These observations have led researchers to investigate whether nonsteroidal anti-inflammatory drugs (NSAIDs) might slow the progression of dementia. Initially, two small studies with the NSAIDs indomethacin (Rogers *et al*, 1993) and diclofenac (Scharf *et al*, 1999) provided preliminary evidence for efficacy over 6 months in patients with AD. However, interpretation of the results from both studies was confounded by high dropout rates, mainly due to gastrointestinal side effects thought to be mediated via inhibition of COX-1 (nonselective NSAIDs inhibit both COX-1 and COX-2) (Warner *et al*, 1999). More recently, three larger randomized, controlled, 1-year clinical studies with the selective COX-2 inhibitors rofecoxib or celecoxib failed to show any effects of treatment on the progression of AD (Sainati *et al*, 2000; Aisen *et al*, 2003; Reines *et al*, 2004). One of these studies included the nonselective NSAID naproxen, which also failed to show any effects (Aisen *et al*, 2003). In addition, the anti-inflammatory agent hydroxychloroquine did not show any benefits in an 18-month trial (Van Gool *et al*, 2001).

A possible explanation for the lack of efficacy in the above studies is that the underlying pathology might be too advanced in patients with an established diagnosis of AD for an anti-inflammatory treatment to alter the course of the disease. Epidemiological evidence indicates that there may

*Correspondence: Dr CR Lines, Merck Research Laboratories, 10 Sentry Parkway, Blue Bell, PA 19422, USA, Tel: +1 484 344 2595, Fax: +1 484 344 4940, E-mail: chris_lines@merck.com

[5]Members of the Rofecoxib Protocol 078 study group are listed in Acknowledgments

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be a critical period, 2 or more years before the onset of dementia, during which exposure to NSAIDs protects against AD (in 't Veld *et al*, 2001). Selective COX-2 inhibitors offer the potential for treatments that may have beneficial protective effects in AD while being well tolerated in long-term use. We therefore conducted a study to determine whether treatment with rofecoxib could delay a diagnosis of AD. Rather than evaluating a general elderly population with a relatively low incidence rate of AD, we sought to recruit elderly patients with mild cognitive impairment (MCI) (Petersen *et al*, 2001a, b). These patients were expected to have an annual AD diagnosis rate of 10–15% *vs* a rate of 1–2% for the general elderly population (Petersen *et al*, 2001a, b). The study also provided the opportunity to gather important placebo-controlled, long-term safety data on rofecoxib in an elderly population.

METHODS

Patients

Patients aged 65 years or older who had completed at least 8 grades of education, and had a reliable informant who could accompany them to each clinic visit, were recruited at 46 study sites in the United States from April 1998 to March 2000. Potentially eligible patients were initially identified by investigators (by any means available) or via a centralized telephone prescreening process (Lines *et al*, 2003). Patients were screened at the study sites to determine if they met all the following criteria for MCI: patient reports memory problem, or informant reports that patient has memory problem; informant reports that patient's memory has declined in the past year; Mini Mental State Exam (MMSE) (Folstein *et al*, 1975) score $\geqslant 24$; Clinical Dementia Rating (CDR) (Morris, 1993) global score $= 0.5$ with memory domain score $\geqslant 0.5$; Blessed Dementia Rating Scale (BDRS) (Morris *et al*, 1988) total score $\leqslant 3.5$, with no part 1 item score > 0.5; Auditory Verbal Learning Test (AVLT) (Schmidt, 1996) total score $\leqslant 37$. The cut score on the AVLT corresponds to a score $\leqslant 1$ standard deviation (SD) below the mean for normal elderly subjects; for the first 6 months of study enrollment, age-adjusted cut scores 1.5 SDs below the means for separate age bands were used (see Appendix A1). Further details of these tests of cognition and function are given in Appendix A1. The CDR assessment was completed on the basis of interviews with the patient and informant by a rater who was blinded to the results of psychometric tests (AVLT and MMSE).

Patients were excluded if they had: dementia; inadequate motor or sensory capacities to comply with testing; a modified Hachinski Ischemic Scale (Rosen *et al*, 1980) score > 4 (to exclude patients whose cognitive impairment may have been related to a vascular condition); a Hamilton Depression Scale (17-item version) (Hamilton, 1960) score > 13 (to exclude patients whose cognitive impairment may have been related to depression); a history of angina or congestive heart failure with symptoms that occurred at rest; uncontrolled hypertension; a history within the past year of myocardial infarction, coronary artery bypass, angioplasty, or stent placement; a history within the past 2 years of stroke, multiple lacunar infarcts, or transient ischemic events; a history within the past 3 months of gastrointestinal bleeding; an expected therapeutic need for chronic NSAID or estrogen replacement therapy during the study. Patients taking NSAIDs on a chronic basis ($\geqslant 7$ days/month for the 2 months prior to study entry), estrogen replacement therapy (excluding topical ointments) within 2 months of study entry, or cholinesterase inhibitors within 1 month of study entry were also excluded. No more than 20% of patients at each study site could be taking vitamin E > 400 IU at the time of study entry. Concomitant use of the above medications during the study was discouraged, but patients who did take them were not discontinued for this reason. Patients who developed a need for cardio-protective doses of aspirin after randomization were permitted to use aspirin $\leqslant 100$ mg/day; clopidogrel was also allowed. Each site received the approval of its local institutional review board to perform the study, and informed consent was obtained from each subject.

Design

This was a randomized, double-blind, placebo-controlled study with parallel groups. After screening, eligible patients were randomly assigned to receive rofecoxib 25 mg once daily or placebo once daily for up to 4 years. Rofecoxib 25 mg was the maximum recommended dose for approved chronic indications (Merck & Co. Inc., 2003; on September 30, 2004, Merck & Co. Inc., announced the voluntary worldwide withdrawal of rofecoxib from the market). Randomization of patients at each study site was determined by a computer-generated allocation schedule and was stratified according to MMSE score (24–26, > 26). The allocation schedule was generated by a statistician at Merck Research Laboratories according to in-house blinding conditions. The rofecoxib and placebo tablets were visually identical. The original intention was that the study would be event-rate driven and would continue until 220 patients had received an AD diagnosis, which it was anticipated would take approximately 2 years. The study was extended to 4 years due to lower than expected AD diagnosis rates. A decision was made in October 2002 to terminate the study in April 2003, 11 months earlier than the scheduled March 2004 termination (ie 4 years after the last patient was enrolled). Based on the diagnosis and discontinuation rates observed as of July 2002, it was estimated that 219 end points should have been observed by April 2003. In fact, only 189 diagnoses of AD had been made at this time, and discontinuation rates had continued to increase. The combination of the low diagnosis rates and increasing discontinuation rates made it unlikely that the target number of events could have been achieved and it was therefore considered that continuation of the study until its planned termination would not have been productive. The decision was made prior to unblinding.

Procedure

After randomization, patients were scheduled to attend the clinic at months 1 and 4, and then every 4 months until the study was completed or the patient was diagnosed with dementia. The study was conducted using an intent-to-treat approach; that is, patients who discontinued treatment, but who had not developed dementia, were asked to return to

the clinic for all remaining visits and assessments. The following assessments were administered at baseline and every 4 months, or at discontinuation from the study: CDR, MMSE, Selective Reminding Test (SRT; see Appendix A1 for details) (Buschke, 1973). The cognitive subscale of the AD Assessment Scale (ADAS-Cog; see Appendix A1 for details) (Rosen *et al*, 1984) was administered at baseline and then every 12 months, or at discontinuation from the study. The BDRS was administered at baseline and at 24, 36, and 48 months, or at discontinuation from the study. Any patient who received a global CDR score $\geqslant 1$ at a routine clinic visit was suspected of having converted to dementia and administered a CT or MRI scan of the brain and any psychometric tests that were not already scheduled for that visit. The patient was continued on treatment and returned for an end point confirmation visit 2 months later, at which time all the psychometric evaluations were repeated. If the global CDR score was still $\geqslant 1$ at this visit, the patient was considered to have reached the end point of dementia and was discontinued from the study (regardless of the outcome of the adjudication process described below). In some cases, a patient was determined by an investigator to have developed dementia despite maintaining a global CDR score of 0.5 at the trigger and confirmation visits, and these patients were also counted as end points and were discontinued from the study. The investigator determined the type of dementia: possible or probable AD according to NINCDS-ADRDA criteria (McKhann *et al*, 1984) or other, for example, vascular dementia. For patients who reached the end point of clinically diagnosed dementia, all relevant data were sent to an independent blinded adjudication committee consisting of three experts. Each adjudicator reviewed the data independently and indicated whether or not they concurred with the investigator's diagnosis. In order to qualify as an event for the primary analysis, a majority decision in $\geqslant 2$ of the three adjudicators that the patient met criteria for possible or probable AD was required (ie $\geqslant 2$ adjudicators classified the event as probable AD, $\geqslant 2$ adjudicators classified the event as possible AD, or one adjudicator classified the event as possible AD and one adjudicator classified it as probable AD).

Any adverse experiences occurring during the study were recorded and rated by the investigator, while still blinded to the treatment that the patient was receiving, as to seriousness (death, life threatening, resulting in persistent or significant disability, resulting in hospitalization, prolonging an existing hospitalization, any other important medical event), drug-relatedness (possibly, probably or definitely drug-related, probably not or definitely not drug-related), and intensity (mild, moderate, or severe). All serious vascular events (including cardiac, peripheral vascular, and cerebrovascular events) and upper gastrointestinal perforations, ulcers, and bleeds were reviewed by independent blinded adjudication committees, who determined if they were confirmed events according to prespecified case definitions (confirmed events) (Bombardier *et al*, 2000; Konstam *et al*, 2001).

Statistical Analysis

The primary efficacy analysis compared the cumulative incidence of possible or probable AD according to NINCDS-ADRDA criteria (McKhann *et al*, 1984); patients with dementia of other cause were censored in the analysis at the time of diagnosis. AD diagnoses confirmed by the end point adjudication committee were the only end points included in the primary analysis. The analysis was based on a Cox proportional hazards model of time-to-event data (based on the initial diagnosis of AD) using an intention-to-treat approach, which included all randomized patients regardless of whether or not they were taking study medication. The model included terms for treatment, region within the United States (North East, South East, South, Midwest, West), and baseline MMSE strata (baseline MMSE score $\leqslant 26$ *vs* >26). Region was intended to be a surrogate for investigating the influence of study site, since there were too few events at individual study sites to include that as a factor. An additional prespecified on-drug analysis was restricted to patients who converted to AD within 14 days of being on study medication.

The calculation for the power statement assumed that the incidence of possible or probable AD over 2 years in the placebo group would be 30% and that the discontinuation rate would be 20%. Based on these considerations and a planned sample size of 520 evaluable patients per group, the study had 90% power to detect a one-third reduction in the incidence of AD in the rofecoxib group *vs* the placebo group with two-tailed $\alpha = 0.05$.

Analyses of prespecified secondary measures (SRT-summed recall score, SRT-delayed recall score, MMSE score, ADAS-Cog score, CDR-sum of boxes score, BDRS score) were based on available data for evaluable patients (ie patients who had a baseline score and at least one postrandomization score). The annual rates of change from baseline to a given time point (slopes), and slope differences between groups, were estimated using an intention-to-treat approach (ie including all data regardless of whether a patient discontinued from therapy or not) and analyses were performed using longitudinal repeated measures models for the comparison, under the assumption that missing data were missing at random (ie ignorable missingness). Additional sensitivity analyses were also performed using a last-observation-carried forward approach, as well as with other models with different assumptions about the missing data structure.

The present study had a broadly similar design (randomized, double-blind, placebo-controlled) and utilized some similar assessments (ADAS-Cog and CDR) to a previous 1-year AD treatment study of rofecoxib 25 mg in patients who had MMSE scores of 14–26 (Reines *et al*, 2004). Since there were overlapping subgroups of patients who had MMSE scores of 24–26 in both studies, indicating a similar level of cognitive impairment despite the difference in diagnosis, we also conducted a *post hoc* analysis to compare change from baseline scores on measures of cognition (ADAS-Cog) and global function (CDR-sum of boxes) in the overlapping subgroups in the two studies. The analysis looked at estimated annual slope differences using the same methods as described above. For the subgroup from the previous AD treatment trial, the annual slope estimates were derived from data over 12 months. For the subgroup from the present study, the annual slope estimates were derived from data over 48 months.

The assessment of tolerability included on-drug adverse experiences with onset up to 14 days after patients stopped taking test medication, and was based on the population of patients who took at least one dose of study medication. Prespecified groupings of adverse experiences (eg the number of patients with one or more adverse experience) were analyzed using Fisher's exact test.

RESULTS

The study profile is shown in the study flowchart (Figure 1). A total of 1457 patients were randomized. The study was terminated after 189 confirmed diagnoses of AD had been made. The median duration of study participation was 115 weeks in the rofecoxib group and 130 weeks in the placebo group. The median duration patients took study medication was 94 weeks in the rofecoxib group and 105 weeks in the placebo group. Estimates of treatment compliance based on counts of the number of tablets in medication bottles at each clinic visit and calculated as ((number of days on therapy/number of days in study) × 100) indicated reasonable compliance; 61.0% of the 725 patients randomized to rofecoxib and 70.8% of the 732 patients randomized to placebo had ⩾80% compliance during the time they were in the study. Approximately 45% of patients discontinued the study prematurely, while 40% completed the study on-drug (including patients who had not completed 48 months of treatment but were still in the study at the time of termination), and 15% completed the study off-drug. Reasons for discontinuation were generally similar across the treatment groups, although there were some small differences; for example, a greater proportion of patients in the placebo group discontinued due to withdrawal of consent (see Figure 1). The time course of discontinuations was similar between the treatment groups over the duration

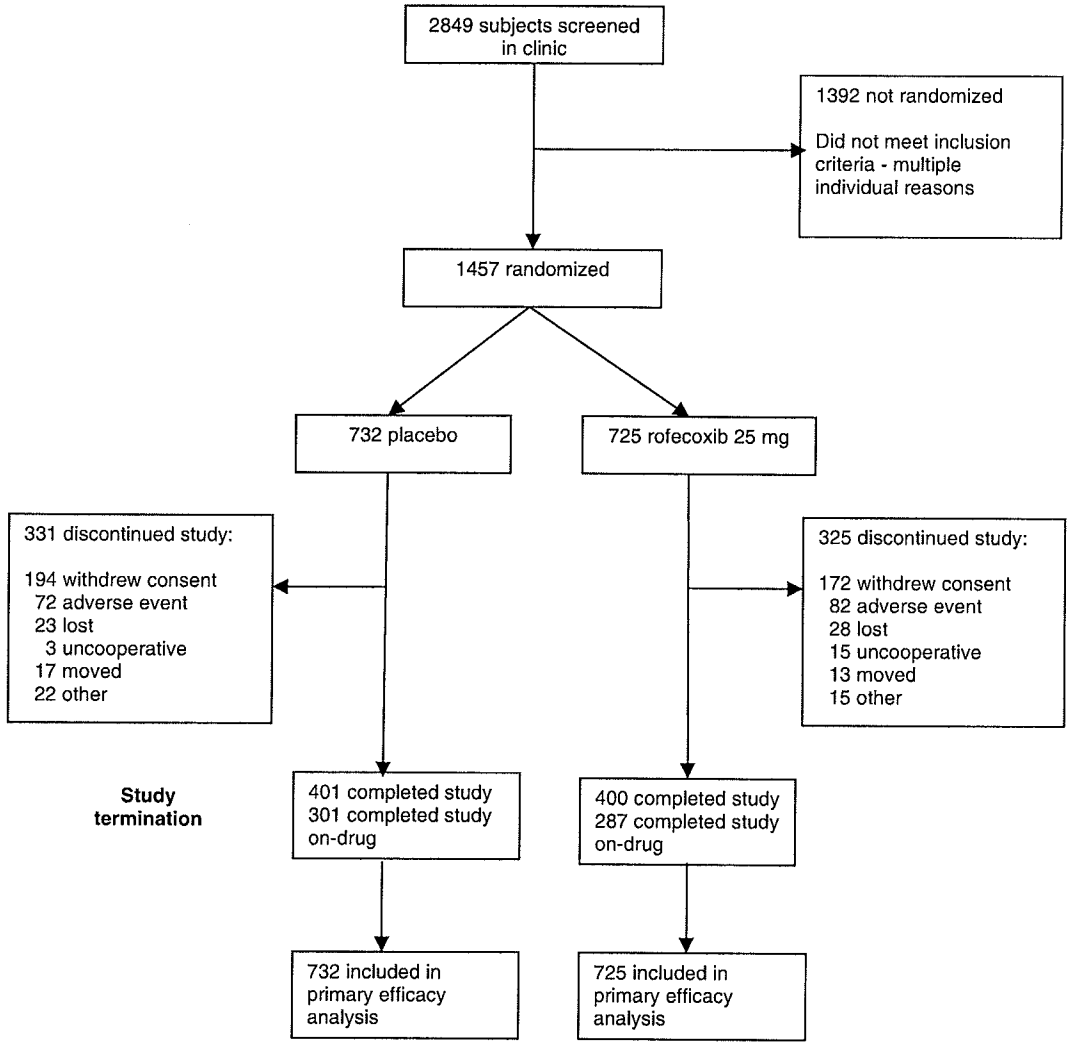

Figure 1 Study flowchart. Patients were prescreened for eligibility before being screened in the clinic. Data on the precise number of subjects prescreened were not collected, but the number was > 17 000. The total who completed the study includes patients who completed 48 months of study participation ($N = 115$ for rofecoxib, $N = 146$ for placebo), patients who were diagnosed with dementia of any cause ($N = 112$ for rofecoxib, $N = 83$ for placebo), patients who completed less than 48 months of study participation but were still in the study at the time it was terminated ($N = 121$ for rofecoxib, $N = 123$ for placebo), and patients who were discontinued because the study site closed ($N = 52$ for rofecoxib, $N = 49$ for placebo).

of the study (data not shown). Of those who discontinued, placebo patients had higher baseline ADAS-Cog scores, indicative of greater cognitive impairment and possibly greater risk of progression to AD, than patients randomized to rofecoxib ($p = 0.034$ in a logistic regression model); the mean baseline ADAS-Cog score was 9.6 in placebo discontinuers *vs* 9.1 in rofecoxib discontinuers. The change from baseline to last evaluation time point scores on the ADAS-Cog, MMSE, and CDR-sum of boxes in the subgroup of discontinued patients who had at least one on-treatment evaluation was similar between the treatment groups (data not shown).

Table 1 shows the baseline demographic characteristics and baseline test scores for randomized patients. The groups were generally comparable with regard to baseline characteristics and test scores. The distribution of secondary diagnoses at baseline was generally similar between the groups. The most common pre-existing medical conditions were hypertension (37.7% in the rofecoxib group and 34.3% in the placebo group) and osteoarthritis (22.2% in the rofecoxib group and 24.3% in the placebo group). The percentages of patients with reported prior use (for any length of time in the 2 months prior to study entry for NSAIDs and estrogen, and 1 month prior to study entry for other drugs) of drugs claimed to have an influence on dementia were generally similar in the rofecoxib *vs* placebo groups for NSAIDs (8.4 *vs* 10.2%), ginkgo (12.6 *vs* 11.2%), statins (14.9 *vs* 13.0%), estrogen (0.4 *vs* 1.4%), and vitamin E >400 IU daily (5.8 *vs* 5.6%). The percentages of patients with reported concomitant use (for any length of time during the study) of drugs claimed to have an influence on dementia were lower in the rofecoxib group than the placebo group for NSAIDs (30.9 *vs* 34.7%) and estrogen (2.6 *vs* 4.8%), higher for cholinesterase inhibitors (11.2 *vs* 8.6%), and similar for statins (24.8 *vs* 24.9%), ginkgo (13.7 *vs* 12.8%), and vitamin E >400 IU daily (7.3 *vs* 7.5%). The median duration of reported concomitant NSAID use was lower in the rofecoxib group (5.6 weeks) than the placebo group (7.4 weeks). The percentages of patients with reported aspirin use in the 2 months prior to the study were 10.1% in the rofecoxib group and 9.0% in the placebo group. The percentages of patients with reported aspirin during the study were 31.7% in the rofecoxib group and 29.9% in the placebo group.

Table 1 Baseline Patient Characteristics

Characteristic	Placebo ($N = 732$)[a]	Rofecoxib 25 mg ($N = 725$)[a]
% Women	31.1%	34.3%
% Family history of AD	29.5%	31.2%
% Apolipoprotein ε4	35.8%	34.6%
Years of education		
11 or less	9.8%	11.2%
12–17	77.7%	74.7%
18 or more	12.4%	13.4%
Mean (SD) years of age	74.8 (6.0)	75.1 (6.0)
Mean (SD) AVLT score[b]	28.6 (5.8)	28.6 (5.7)
Mean (SD) MMSE score[c]	27.3 (1.7)	27.4 (1.7)
Mean (SD) CDR-sum of boxes score[d]	1.4 (0.8)	1.4 (0.8)
Mean (SD) ADAS-Cog score[e]	9.4 (3.8)	9.2 (4.0)
Mean (SD) SRT-summed recall score[f]	33.6 (9.1)	33.4 (9.0)
Mean (SD) SRT-delayed recall score[g]	4.5 (2.4)	4.4 (2.5)
Mean (SD) BDRS score[h]	1.1 (0.7)	1.1 (0.8)

[a]Sample sizes varied for some measures due to missing data. For apolipoprotein genotyping, sample sizes were 669 for placebo and 652 for rofecoxib.
[b]Auditory Verbal Learning Test. Range = 0–75; lower score indicates greater impairment.
[c]Mini Mental State Exam. Range = 0–30; lower score indicates greater impairment.
[d]Clinical Dementia Rating. Range = 0–18; higher score indicates greater impairment.
[e]Cognitive subscale of the AD Assessment Scale. Range = 0–70; higher score indicates greater impairment.
[f]Selective Reminding Test (immediate recall component). Range = 0–72; lower score indicates greater impairment.
[g]Selective Reminding Test (delayed recall component). Range = 0–12; lower score indicates greater impairment.
[h]Blessed Dementia Rating Scale. Range = 0–17; higher score indicates greater impairment.

A total of 195 investigator diagnoses of dementia (190 diagnoses of possible or probable AD and five diagnoses of non-AD dementia) were evaluated by the end point adjudication committee. The primary end point of clinically diagnosed AD included the 189 patients who were confirmed to have developed possible or probable AD by ⩾2 members of the committee. Two events adjudicated to be non-AD dementia, two events adjudicated to be nondementia, and two events in which there was no agreement between adjudicators (one adjudicator classified the event as AD, one classified it as non-AD dementia, and one classified it as nondementia) were not included as end points in the primary analysis. In the 189 patients with adjudicator-confirmed clinically diagnosed AD, global CDR scores at the last clinic visit were 0.5 (questionable dementia) in 21 patients, one (mild dementia) in 159 patients, two (moderate dementia) in eight patients, and three (severe dementia) in one patient.

In the rofecoxib group, 107 of 725 (14.8%) patients had clinically diagnosed AD over the 4-year study period *vs* 82 of 732 (11.2%) placebo patients over 4 years. The estimated hazard ratio (rofecoxib : placebo), adjusting for the effects of baseline MMSE stratum and region, was 1.46 (95% CI: 1.09, 1.94), which was statistically significant ($p = 0.011$, Wald χ^2 test) in favor of placebo. The treatment-by-time interaction was not significant ($p = 0.260$), indicating that the proportional hazards assumption was reasonably met. Baseline MMSE stratum (score ⩽26; score >26) had a highly statistically significant effect on outcome with an estimated hazard ratio of 3.23 (95% CI: 2.42, 4.32) ($p < 0.001$), indicating that patients in the lower stratum were much more likely to progress to AD irrespective of treatment assignment. Treatment effects were consistent across MMSE stratum levels, as well as geographic regions. Kaplan–Meier estimated proportions of patients with clinically diagnosed AD at 4-month increments are shown in Table 2. A separation in event rates between treatment groups was evident at the earliest time point (4 months), a gap that was

Table 2 Kaplan–Meier Estimated Proportions of Clinically Diagnosed AD at 4-Month Intervals

	Placebo		Rofecoxib	
Month after treatment	N^a	Estimated proportion	N^a	Estimated proportion
0	732	0.0	725	0.0
4	707	0.3	682	0.9
8	666	1.4	642	3.1
12	625	3.1	582	5.1
16	581	4.2	535	7.4
20	528	6.4	486	9.2
24	458	9.2	415	12.3
28	390	10.3	344	14.2
32	359	11.0	311	15.7
36	310	12.5	258	17.9
40	240	13.7	214	19.3
44	177	15.6	159	21.0
48	86	17.4	68	23.0

[a]Number of patients at risk (number who had not been censored nor had an event up to, but not including, that time point).

maintained through the last follow-up time point of 48 months as shown by the nonsignificant p-value for the test of proportionality of treatment-specific hazard rates (see above). Estimated annual diagnosis rates were 6.4% (95% CI: 5.3%, 7.7%) in the rofecoxib group and 4.5% (95% CI: 3.6%, 5.6%) in the placebo group. The above analysis included patients whether or not they were taking study medication. In the prespecified analysis looking at events that occurred on-drug ($N=723$ for rofecoxib and $N=728$ for placebo), there was no evidence for an increased hazard ratio (1.49 (95% CI: 1.08, 2.05), $p=0.014$) compared with the hazard ratio of 1.46 observed in the primary analysis, which included patients who had stopped taking study treatment.

To further explore the unexpected finding favoring placebo in the primary analysis, we conducted a *post hoc* analysis to adjust for factors that showed an effect, at a significance level of $p<0.10$, on progression to AD. Those factors correlated with greater likelihood of progression to AD were lower baseline MMSE score stratum (24–26), female gender, age >75, and prior ginkgo use. Factors associated with a decreased risk of progressing to AD were longer duration of concomitant NSAID use, and concomitant use of statins. In the analysis that adjusted for these factors, the statistical significance of the hazard ratio was reduced (1.31 (95% CI: 0.98, 1.75) $p=0.065$). Presence of the apolipoprotein ε4 allele was a risk factor but was not included in the model because information was missing for a substantial proportion of patients. Based on a significance level of $p<0.10$, family history of AD did not appear to be a risk factor in this study.

Because rofecoxib and other NSAIDs can cause small mean increases in blood pressure (Gertz *et al*, 2002; Schwartz *et al*, 2002), and there is some evidence to suggest that increased blood pressure might be associated with an increased risk of dementia (Skoog, 1997; Birkenhager *et al*, 2001), we also performed two *post hoc* analyses to evaluate whether the rofecoxib : placebo risk ratio for diagnosis of AD increased as a function of increased blood pressure change. In the first analysis, change from baseline in mean arterial blood pressure (defined as (2 × diastolic blood pressure + systolic blood pressure)/3) at month 4 was calculated for each patient. The rofecoxib : placebo odds ratios for diagnosis of AD were then calculated for three categories of patients: those with no change or a decrease (odds ratio = 1.43), those with an increase ⩽5 mmHg (odds ratio = 1.18), and those with an increase >5 mmHg (odds ratio = 1.47). The Breslow–Day test of homogeneity of the odds ratios across categories indicated no significant differences ($p=0.895$). In the second *post hoc* analysis, we looked at a predefined limit of change in systolic blood pressure, which was prespecified as a postrandomization value that was ⩾180 mmHg and showed a ⩾20 mmHg increase from baseline. The rofecoxib : placebo hazard ratios for diagnosis of AD were similar in those patients who did not meet the predefined limit of change criteria (hazard ratio = 1.42 (95% CI: 1.06, 1.92)), compared with those who did meet the criteria (hazard ratio = 1.53 (95% CI: 0.49, 4.81)).

The least squares mean scores for the secondary end points of cognition and function at 1-year intervals using the repeated measures models approach described in Methods are summarized in Table 3. The prespecified analysis looked at the estimated annual slope difference (placebo minus rofecoxib) for each measure based on data over the entire 4-year period. In contrast to the primary end point, there were no significant differences between treatment groups on estimated annual slope differences for SRT-summed recall score (slope difference = 0.026 (95% CI: −0.307, 0.359), $p=0.878$), SRT-delayed recall score (slope difference = −0.012 (95% CI: −0.104, 0.081), $p=0.806$), MMSE score (slope difference = −0.002 (95% CI: −0.095, 0.090), $p=0.959$), ADAS-Cog score (slope difference = −0.098 (95% CI: −0.287, 0.091), $p=0.311$), or BDRS score (slope difference = 0.079 (95% CI: −0.081, 0.238), $p=0.333$). The slope difference for the CDR-sum of boxes score showed a nonsignificant trend in favor of placebo (slope difference = −0.068 (95% CI: −0.139, 0.002), $p=0.058$); this would be expected to be highly correlated with the primary end point since the global score on the CDR was used to trigger the diagnosis of AD. Additional sensitivity analyses for the secondary end points were also performed using a last-observation-carried forward approach. The results, indicating a lack of treatment differences, were similar across these analyses, which were based on different assumptions about the missing data structure.

The *post hoc* analysis comparing test scores in overlapping subgroups of patients with MMSE scores of 24–26 in the present study (the subgroup with the worst prognosis) and a previous AD treatment study (Reines *et al*, 2004) included a total of 405 patients (rofecoxib $N=189$, placebo $N=216$) from the present MCI study, and 205 patients (rofecoxib $N=91$, placebo $N=114$) from the previous AD treatment study. There was no consistent evidence of a differential treatment effect of rofecoxib in the

Table 3 Secondary End Points: Least Squares Mean (Standard Error) Scores with Difference and 95% Confidence Interval

Measure	1-Year			2-Year			3-Year			4-Year		
	Placebo[a] ($N^c = 659$)	Rofecoxib[a] ($N^c = 624$)	Difference[a,b] (95% CI)	Placebo[a] ($N^c = 509$)	Rofecoxib[a] ($N^c = 460$)	Difference[a,b] (95% CI)	Placebo[a] ($N^c = 344$)	Rofecoxib[a] ($N^c = 302$)	Difference[a,b] (95% CI)	Placebo[a] ($N^c = 195$)	Rofecoxib[a] ($N^c = 176$)	Difference[a,b] (95% CI)
SRT-summed	35.7 (0.3)	35.6 (0.4)	−0.2 (−1.1,0.7)	36.5 (0.4)	35.7 (0.4)	−0.8 (−1.9,0.3)	36.1 (0.5)	35.7 (0.5)	−0.3 (−1.7,1.1)	36.0 (0.7)	35.6 (0.7)	−0.4 (−2.3,1.5)
SRT-delayed	4.8 (0.1)	4.7 (0.1)	−0.0 (−0.3,0.2)	4.9 (0.1)	4.8 (0.1)	−0.1 (−0.5,0.2)	4.9 (0.1)	4.7 (0.1)	−0.2 (−0.6,0.1)	4.5 (0.2)	4.5 (0.2)	0.1 (−0.5,0.6)
ADAS-Cog	8.9 (0.1)	9.3 (0.1)	0.3 (−0.0,0.7)	9.4 (0.2)	9.7 (0.2)	0.3 (−0.3,0.8)	9.8 (0.3)	10.1 (0.3)	0.3 (−0.4,1.0)	10.3 (0.3)	10.7 (0.3)	0.3 (−0.4,1.1)
MMSE	27.3 (0.1)	27.2 (0.1)	−0.1 (−0.3,0.1)	27.2 (0.1)	27.3 (0.1)	0.1 (−0.2,0.4)	27.1 (0.1)	27.1 (0.1)	−0.1 (−0.4,0.3)	26.7 (0.2)	26.5 (0.2)	−0.2 (−0.7,0.3)
CDR-sum of boxes	1.5 (0.0)	1.6 (0.0)	0.1 (−0.0,0.2)	1.8 (0.1)	2.0 (0.1)	0.2 (−0.0,0.4)	2.1 (0.1)	2.2 (0.1)	0.2 (−0.0,0.4)	2.2 (0.1)	2.4 (0.1)	0.2 (−0.0,0.5)
BDRS	—	—	—	1.4 (0.1)	1.4 (0.1)	0.0 (−0.1,0.2)	1.4 (0.1)	1.5 (0.1)	0.1 (−0.1,0.3)	1.4 (0.1)	1.6 (0.1)	0.1 (−0.1,0.4)

[a]Results based on repeated measures model.
[b]Difference is rofecoxib−placebo.
[c]Sample size (number of patients with any secondary efficacy measure by time point).
See Table 1 for key to test abbreviations and interpretation of direction of scores. 1-year data are not provided for the BDRS because it was not scheduled to be administered until the 2-year time point.

overlapping subgroups in the two studies. The estimated annual slope difference for the ADAS-Cog score was −0.240 (95% CI: −0.736, 0.255), $p = 0.340$ in the MMSE 24–26 subgroup from the present study, and 0.447 (95% CI: −1.284, 2.178), $p = 0.611$ in the MMSE 24–26 subgroup from the previous AD treatment study. The estimated annual slope difference for the CDR-sum of boxes score was −0.180 (95% CI: −0.350, −0.009), $p = 0.039$ in the MMSE 24–26 subgroup from the present study, and −0.053 (95% CI: −0.560, 0.454), $p = 0.837$ in the MMSE 24–26 subgroup from the previous AD treatment study.

A total of 1451 patients were included in the on-drug safety analysis (723 in the rofecoxib group and 728 in the placebo group). There were slightly fewer patients in the safety analysis than in the efficacy analysis because six patients who never took study medication were excluded from the safety analysis. The adverse experience profile is summarized in Table 4. The rofecoxib and placebo groups were similar with regard to the percentages of patients with any adverse experience, any serious adverse experience, and who discontinued treatment due to an adverse experience. There was a significant increase for rofecoxib in the number of patients with adverse experiences that were considered possibly, probably, or definitely drug-related by the investigators. The most common drug-related adverse experiences are shown in Table 4. There was no particular individual adverse experience that contributed to the overall difference between rofecoxib and placebo for drug-related adverse experiences, and relatively few patients discontinued study treatment due to drug-related adverse experiences (58 or 8.0% for rofecoxib and 41 or 5.6% for placebo). The number of patients with confirmed upper gastrointestinal perforations, ulcers, or bleeds was 14 in the rofecoxib group and four in the placebo group. A total of 39 deaths occurred in patients who were taking study treatment or from fatal adverse events that started within 14 days of the last dose (24 or 3.3% for rofecoxib and 15 or 2.1% for placebo). Patients died from a range of causes that were consistent with expectations for an elderly population, and there was no specific pattern as to the cause of death in either treatment group. The only specific fatal adverse events with more than one patient per treatment group were myocardial infarction (four patients on rofecoxib and three on placebo), cardiac arrest (two patients on rofecoxib and none on placebo), pneumonia (two patients on rofecoxib and none on placebo), and renal failure (one patient on rofecoxib and two on placebo). (An individual patient may have had more than one adverse event associated with death.) Off-drug follow-up mortality data were available for less than half of the patients ($N = 356$ for rofecoxib, $N = 307$ for placebo); the median duration of off-drug follow-up in these patients was 29 weeks in the rofecoxib group and 20 weeks in the placebo group. There were an additional 22 deaths in the off-drug period (17 in patients assigned to rofecoxib and five in patients assigned to placebo); 12 of these (11 in the rofecoxib group and one in the placebo group) occurred more than 48 weeks after treatment discontinuation. Compared with the on-drug period, there were an additional five patients with off-drug myocardial infarction fatal adverse events (five rofecoxib, none placebo), and an additional two patients with each of cardiac arrest (two rofecoxib, none placebo), pneumonia

Table 4 Summary of Clinical Adverse Experiences

Number (%) of patients with	Placebo ($N=728$)	Rofecoxib 25 mg ($N=723$)
⩾1 adverse experience	670 (92.0%)	651 (90.0%)
⩾1 drug-related adverse experience[a]	173 (23.8%)	211 (29.2%)*
Serious adverse experience[b]	236 (32.4%)	217 (30.0%)
Discontinued treatment due to adverse experience	147 (20.2%)	156 (21.6%)
Most common drug-related adverse experiences[c]		
Diarrhea	16 (2.2%)	11 (1.5%)
Dyspepsia	17 (2.3%)	24 (3.3%)
Nausea	15 (2.1%)	14 (1.9%)
Dizziness	14 (1.9%)	18 (2.5%)
Peripheral edema	11 (1.5%)	23 (3.2%)
Increased blood pressure	14 (1.9%)	16 (2.2%)
Hypertension	25 (3.4%)	29 (4.0%)

[a]Experiences that were rated as possibly, probably, or definitely drug-related by the investigator.
[b]Death, cancer, any adverse event that was life threatening, resulted in persistent or significant disability, resulted in or prolonged an existing hospitalization, or any other important medical event.
[c]Incidence >2.0% in either treatment group.
*Significantly different from placebo, $p<0.05$.

(two rofecoxib, none placebo), and renal failure (two rofecoxib, none placebo) off-drug fatal adverse events. The number of patients with confirmed serious thrombotic vascular events on-drug was similar in the two groups (38 in the rofecoxib group and 36 in the placebo group). There were a total of six patients with confirmed ischemic strokes and one patient with hemorrhagic stroke in the rofecoxib group compared to 13 patients with confirmed ischemic strokes and two patients with hemorrhagic strokes in the placebo group. Thirteen patients in the rofecoxib group had confirmed nonfatal myocardial infarctions *vs* 10 in the placebo group.

DISCUSSION

In this 4-year study of 1457 patients with MCI, there was no evidence that rofecoxib delayed a diagnosis of AD. A treatment difference in favor of placebo was observed on the primary end point of time to clinically diagnosed AD. This finding was not confirmed by secondary measures of cognition (ADAS-Cog, SRT, MMSE) or global function (BDRS, CDR-sum of boxes), which found no statistically significant or clinically meaningful differences between treatment groups. The possibility that rofecoxib might be inferior to placebo was also not supported by data from two large previous AD treatment studies, which included patients with an MMSE score up to 26 (thereby partly overlapping with patients in the present study who had an MMSE score ⩾24), and found that rofecoxib had no significant effect on the progression of cognitive or functional decline (Aisen *et al*, 2003; Reines *et al*, 2004).

Given the unexpected nature of the primary finding, it was thought important to compare the present results with those from an independent database. We therefore conducted a *post hoc* analysis of test scores in subgroups of patients with MMSE scores of 24–26 in the present study (ie those patients who were most likely to receive a diagnosis of AD) and in a previous AD treatment study (Reines *et al*, 2004). These studies had similar designs and utilized similar assessments. Since MCI is hypothesized to be on a continuum of cognitive impairment ranging from very mild impairment (ie MCI) through mild, moderate, and severe dementia, it is plausible that many patients with MMSE scores of 24–26 in the two studies may have been biologically similar, even though one group was diagnosed with dementia and the other was not. There was no evidence to suggest differences between treatments on assessments of cognition (ADAS-Cog) in this subgroup in either study. In the subgroup analysis from the present study, there was a significant difference between treatments for the CDR-sum of boxes score. This finding was not surprising given that the global score on the CDR was the trigger for diagnosing AD. Indeed, because of its close relation with the primary end point of AD diagnosis, the CDR sum-of boxes score can be viewed as a surrogate for the primary end point. There was no difference between rofecoxib and placebo on the CDR sum-of-boxes score in the subgroup analysis from the previous AD treatment study.

An intriguing observation in the present study was that the separation between treatment groups in rates of diagnosis of AD was apparent from 4 months (the earliest time point assessed) but did not increase over time, raising the possibility that there may have been an imbalance between the groups at baseline. Although there was no clear evidence of a major imbalance for measured baseline variables, including severity of impairment, the model which adjusted for covariates that were risk factors for receiving a diagnosis of AD showed the smallest treatment effect and was not statistically significant. The finding that the treatment difference was not further increased in the analysis restricted to the on-drug population was also not supportive of a true treatment effect.

Since this is the first report of a completed randomized controlled study examining progression to an AD diagnosis in MCI patients, it is important to consider aspects of the design or conduct of the study that could have had an influence on the results. An obvious concern in a study with a long duration is that differential discontinuation rates may have influenced the results. A discontinuation rate of approximately 45% occurred over the course of the study and only 40% of patients completed the study on-drug. These findings are not surprising given the duration of the study and the elderly population being investigated. Overall discontinuation rates, time course of discontinuations, and change from baseline test scores at the time of discontinuation were similar between the treatment groups, although there were some small differences between the groups with regard to specific reasons for discontinuation; for example, a greater proportion of patients in the placebo group discontinued due to withdrawal of consent. The relatively high proportion of patients who withdrew consent may have been a consequence of the fact that patients were required to sign an additional consent form after 2 years,

owing to the study being extended beyond the original timeline, and were unwilling to go beyond their initial commitment. There was also some evidence that patients who discontinued from the placebo group were more impaired at baseline on the ADAS-Cog than patients who discontinued from the rofecoxib group. If the more impaired patients who discontinued were at higher risk of conversion to AD, then differential dropouts may have had an influence on the results.

Another notable observation in the present study was that the overall annual AD diagnosis rate of 5–6% was lower than the anticipated 10–15% annual diagnosis rate reported in previous observational or natural history studies (Petersen *et al*, 2001a, b). There are several possible explanations for this disparity. Firstly, the diagnosis of MCI is heavily dependent on clinical judgment (Petersen, 2003), and the particular tests and score thresholds used to provide objective evidence of memory impairment are not standardized (Chertkow, 2002; Petersen, 2003). It is therefore possible that the population of patients included in the study was more heterogenous than, or different from, MCI populations previously described. Indeed, the population contained a lower proportion of women and patients with the apolipoprotein ε4 allele than others have reported (Petersen *et al*, 1999; Jack *et al*, 1999; Morris *et al*, 2001). Another possible contributing factor to the low diagnosis rates was the change in the cut-score criteria on the AVLT (the memory test used to provide objective evidence of memory impairment) during the study. After 6 months of enrollment, the cutoff was changed from age-adjusted 1.5 SD cut scores to a single cut score 1 SD below the mean for normal elderly subjects (see Appendix A1). We retrospectively looked at diagnosis rates in patients who met the original 1.5 SD criteria ($N=203$ for rofecoxib and $N=203$ for placebo) and the estimated annual diagnosis rates (11.3% for rofecoxib and 8.3% for placebo) were more in line with previous data, although still lower than anticipated for the placebo group. Finally, the fact that the MCI concept was relatively new at the time the study was initiated, along with the source of the patients (advertising campaigns as well as direct clinical referrals), may have also resulted in greater heterogeneity with regard to underlying etiology in the recruited patients.

To summarize the efficacy data, the unexpected primary finding in the present study suggesting that rofecoxib might accelerate the rate of AD diagnosis in patients with MCI was not supported by data on secondary measures in the same study, nor by other data from previous studies that assessed cognitive and functional decline in AD patients (Aisen *et al*, 2003; Reines *et al*, 2004). These observations suggest that the finding may not be indicative of a true treatment effect. If not a true effect, then the most likely explanations are a chance occurrence, or an imbalance between the treatment groups that existed at baseline or arose during the study due to differential discontinuations.

We cannot exclude the possibility that rofecoxib might accelerate conversion of MCI patients to AD, although this possibility would be at variance with the prior epidemiological and clinical data cited above, as well as the body of experimental evidence suggesting that COX-2 inhibition might attenuate neuronal death in several disease states including Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis, in addition to AD (Andreasson *et al*, 2001; Jain *et al*, 2002; Xiang *et al*, 2002; Giovannini *et al*, 2003; Scali *et al*, 2003; Teismann *et al*, 2003; Qin *et al*, 2003; Consilvio *et al*, 2004; Rose *et al*, 2004). The early onset of the treatment difference (and the lack of progressive widening of the difference over the course of the trial) further argues against a direct effect of rofecoxib on the underlying pathophysiology in AD. It could be speculated that the apparent increased conversion to AD might be secondary to a non-AD-specific aspect of rofecoxib's biological effects. For example, rofecoxib and other NSAIDs can cause small mean increases in blood pressure (Gertz *et al*, 2002; Schwartz *et al*, 2002). It has been suggested that increased blood pressure might be associated with an increased risk of dementia, although the evidence is inconsistent (Skoog, 1997; Birkenhager *et al*, 2001). We investigated this possibility by performing *post hoc* analyses to determine if the rofecoxib:placebo risk ratio for diagnosis of AD increased as a function of increased blood pressure change. There was no evidence for such a relationship. A proposed mechanism by which increased blood pressure could lead to dementia is due to an increased risk for cardiovascular adverse events such as strokes or infarcts. As noted below, there was no evidence for an increase in these types of events in patients taking rofecoxib. Furthermore, CT or MRI brain scans were performed in all patients diagnosed with dementia, to exclude the possibility of vascular dementia.

In addition to evaluating efficacy, the present study provided important placebo-controlled data on the safety of rofecoxib 25 mg over periods of up to 4 years in an elderly population. The median duration of exposure to study medication was approximately 2 years. The mean age of patients was 75 years and approximately 50% were at least 75 years old. Previous safety data on rofecoxib come largely from osteoarthritis studies looking at a relatively younger population with a mean age around 60 years (Langman *et al*, 1999; Reicin *et al*, 2002). Rofecoxib was generally well tolerated by the elderly patients in the study, consistent with results from prior clinical studies in osteoarthritis (Langman *et al*, 1999; Reicin *et al*, 2002) and AD (Reines *et al*, 2004). The overall incidence of adverse experiences, serious adverse experiences, and discontinuations due to adverse experiences were similar or only slightly increased for rofecoxib *vs* placebo. Not surprisingly, there was an increase in the number of adverse experiences thought to be drug-related by the investigators for rofecoxib *vs* placebo, but relatively few patients discontinued treatment due to these adverse experiences. The increase was mainly due to small increases in adverse experiences known to be associated with NSAID use and previously reported for rofecoxib such as dyspepsia, hypertension, and peripheral edema. Despite the high mean age of the study population, few patients had confirmed upper gastrointestinal perforations, ulcers, or bleeds, although there were numerically more in the rofecoxib group than in the placebo group. Elderly patients are at increased risk for serious vascular events. Rofecoxib did not appear to increase the risk in this study, since the number of confirmed serious thrombotic vascular events was similar in each treatment group. The procedure used for determining confirmed cardiovascular events, such as heart attack and stroke, was the same as for the recent

3-year APPROVe (Adenomatous Polyp Prevention on Vioxx) study in 2586 patients (62% male, mean age 59 years) with a history of colorectal adenomas, which found an approximately two-fold increased relative risk for these events in patients taking rofecoxib *vs* placebo, beginning after 18 months of treatment (Bresalier *et al*, 2004). The reason for the discrepancy between the present findings and those from APPROVe is unclear. No striking differences were noted between the treatment groups with regard to other, nonserious, vascular adverse events in the present study. The total number of deaths and causes of death on-drug were consistent with expectations for an elderly population, and there was no specific pattern as to the cause of death in either treatment group. The off-drug mortality data are difficult to assess since follow-up information was available for less than half the patients, there was an imbalance in the extent of follow-up data between the two groups, and the majority of deaths occurred more than 48 weeks after treatment had been discontinued.

In conclusion, the finding that rofecoxib did not delay a diagnosis of AD in the present study, or slow the progression of AD in previous studies (Aisen *et al*, 2003; Reines *et al*, 2004), suggests that inhibition of COX-2 is not a useful therapeutic approach in AD. It is possible that nonselective NSAIDs may still be found to have beneficial effects in treating or delaying the progression of symptoms in AD, due to factors other than COX-2 inhibition. However, the only long-term results from an adequately designed study with a nonselective NSAID (naproxen) available to date are not encouraging (Aisen *et al*, 2003). Trials evaluating alternative, non-anti-inflammatory, approaches to delaying a diagnosis of AD in patients with MCI are underway (Petersen, 2003).

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Participating investigators in the Rofecoxib Protocol 078 study group were as follows: Piero Antuono, Froedtert Lutheran Memorial Hospital, Milwaukee, WI; Barry Baumel, Baumel-Eisner Neuromedical Institute, Miami, FL; Karen Bell, Columbia University, New York, NY; B Ian Blatt, Southeastern Pennsylvania Medical Institute, Havertown, PA; Martin Conway, Lovelace Scientific Resources Inc., Albuquerque, NM; Howard S Cummins, The Institute for Advanced Clinical Research, Elkins Park, PA; Mary J Derbenwick, Atlantic Institute of Clinical Research, Daytona Beach, FL; P Murali Doraiswamy, Duke University Medical Center, Durham, NC; Eugene DuBoff, Summit Research Network Inc., Denver, CO; Keith Edwards, Bennington, VT; Larry Eisner, Baumel-Eisner Neuromedical Institute, Jay Ellis, Neuroscience Research, Pittsfield, MA; Donald England, Radiant Research, Eugene, OR; James Ferguson, Pharmacological Research Corp, Salt Lake City, UT; Jerome Goldstein, San Francisco Headache Clinic, San Francisco, CA; James Hartford, Summit Research Network, Cincinnati, OH; Jon Heiser, Pharmacology Research Institute, Northridge, CA; Daniel Hier, University of Illinois at Chicago, Chicago, IL; Bruce Hodges, Midwest Center for Clinical Studies, Kansas City, MO; Richard Holub, Neurological Associates of Albany, Albany, NY; Lawrence R Jenkyn, Dartmouth-Hitchcock Medical Center, Lebanon, NH; F Cleveland Kinney, University of Alabama at Birmingham, Birmingham, AL; Louis Kirby, Pivotal Research Centers, Peoria, AZ; Peter D Londborg, Seattle, WA; Elliott Mancall, Headache Center, Philadelphia, PA; David Margolin, Margolin Brain Institute, Fresno, CA; James McCarthy, Comprehensive NeuroScience Inc., South Yarmouth, MA; William McEntee, Comprehensive NeuroScience Inc., Sarasota, FL; Margarita Nunez, Comprehensive NeuroScience Inc., St Petersburg, FL; Jorg Pahl, Pahl Brain Associates PC, Oklahoma City, OK; Anton Porsteinsson, University of Rochester, Rochester, NY; Eric Pfeiffer, USF Suncoast Gerontology Center, Tampa, FL; Norman Relkin, New York Presbyterian Hospital, New York, NY; Joel S Ross, Long Branch, NJ; Barry Rovner, Philadelphia, PA; Ward Tolbert Smith, Summit Research, Portland, OR; John A Stoukides, Comprehensive NeuroScience Inc., East Providence, RI; Joseph Tate, DeKalb-Gwinnett, OB/GYN, PC, Norcross, GA; Stephen Thein Jr, Pacific Research Network, San Diego, CA; Harvey Tilker, Four Rivers Clinical Research Center, Paducah, KY; Jerry R Tindel, Radiant Research, Austin, TX; Jack Tomlinson, Alzheimer's Diagnostic Clinic, Wichita Falls, TX; Richard Weisler, Raleigh, NC; Jeanette Wendt, Neurological Associates of Tucson, Tucson, AZ; Kerri Wilks, Innovative Medical Research, Towson, MD; Parvaneh Zolnouni, California Clinical Trials, Beverly Hills, CA.

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APPENDIX A1

Details of Tests Used

MMSE (Folstein *et al*, 1975): This is a brief test of overall cognitive function (range of scores = 0–30, lower score indicates greater impairment). Patients who scored <24 were excluded because of the possibility that they might have dementia.

AVLT (Schmidt, 1996): This is a 15-item, five-trial, word recall test (range of scores = 0–75, lower score indicates greater impairment). The interference trial and delayed recall trial, which usually follow the initial five-trial learning phase, were not administered. Patients had to score $\leqslant 37$ words recalled correctly over five trials as objective evidence of memory impairment. This corresponds to a score $\leqslant 1$ SD below the mean for normal elderly subjects aged 65–69 (Ivnik *et al*, 1990). For the first 6 months of study enrollment, age-adjusted cut scores 1.5 SDs below the means for separate age bands were used (eg there was a cut score for those aged 67–69, another cut score for those aged 70–72, etc), but the criteria were modified after 6 months due to concerns that the original criteria may have been too strict and were conflicting with investigator's clinical judgment (see Petersen, 2003), resulting in low enrollment.

CDR (Morris, 1993): This is a global dementia rating scale, based on a semistructured interview with the patient and an informant. Patients are rated on dementia severity (0 = none, 0.5 = questionable dementia, 1 = mild dementia, 2 = moderate dementia, 3 = severe dementia) in six domains or 'boxes' (memory, orientation, judgment and problem solving, community affairs, home and hobbies, personal care). The global CDR score (range = 0–3, higher score indicates greater impairment) was determined according to a computerized version of the published scoring algorithm (http://www.biostat.wustl.edu/~adrc/cdrpgm/index.html). The sum of scores on the individual boxes was also calculated (range = 0–18, higher score indicates greater impairment). Patients had to have a global CDR score of 0.5 (questionable dementia) with a score of at least 0.5 in the memory domain to qualify for study entry.

ADAS-Cog (Rosen *et al*, 1984): This consists of 11 performance-based subtests of cognition, including measures of memory and praxis. The error scores on each subtest are added to give the total score (range = 0–70, higher score indicates greater impairment).

SRT (Buschke, 1973): This is a 12-item, six-trial, word list recall test with selective reminding of words not correctly recalled on the immediately preceding trial. Following the sixth trial, there is a 5 min delay, after which the patients are again asked to recall the list of 12 words. Two scores were derived—the sum of the number of words recalled over each of the six selective reminding trials (SRT-summed recall: range = 0–72, lower score indicates greater impairment) and the number of words recalled after the 5 min delay (SRT-delayed recall; range = 0–12, lower score indicates greater impairment).

BDRS (Morris *et al*, 1988): The version from the CERAD battery was used. This is an informant-based rating of a patient's ability to perform activities of daily living such as household tasks (Part 1) and self-care (Part 2) (range of scores = 0–17, higher score indicates greater impairment). Patients who scored >3.5 with any Part 1 item score >0.5 (indicating severe impairment) were excluded because of the possibility of dementia.

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EXHIBIT B

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Vitamin E and Donepezil for the Treatment of Mild Cognitive Impairment

Ronald C. Petersen, Ph.D., M.D., Ronald G. Thomas, Ph.D., Michael Grundman, M.D., M.P.H., David Bennett, M.D., Rachelle Doody, M.D., Ph.D., Steven Ferris, Ph.D., Douglas Galasko, M.D., Shelia Jin, M.D., M.P.H., Jeffrey Kaye, M.D., Allan Levey, M.D., Ph.D., Eric Pfeiffer, M.D., Mary Sano, Ph.D., Christopher H. van Dyck, M.D., and Leon J. Thal, M.D., for the Alzheimer's Disease Cooperative Study Group*

ABSTRACT

BACKGROUND

Mild cognitive impairment is a transitional state between the cognitive changes of normal aging and early Alzheimer's disease.

METHODS

In a double-blind study, we evaluated subjects with the amnestic subtype of mild cognitive impairment. Subjects were randomly assigned to receive 2000 IU of vitamin E daily, 10 mg of donepezil daily, or placebo for three years. The primary outcome was clinically possible or probable Alzheimer's disease; secondary outcomes were cognition and function.

RESULTS

A total of 769 subjects were enrolled, and possible or probable Alzheimer's disease developed in 212. The overall rate of progression from mild cognitive impairment to Alzheimer's disease was 16 percent per year. As compared with the placebo group, there were no significant differences in the probability of progression to Alzheimer's disease in the vitamin E group (hazard ratio, 1.02; 95 percent confidence interval, 0.74 to 1.41; P=0.91) or the donepezil group (hazard ratio, 0.80; 95 percent confidence interval, 0.57 to 1.13; P=0.42) during the three years of treatment. Prespecified analyses of the treatment effects at 6-month intervals showed that as compared with the placebo group, the donepezil group had a reduced likelihood of progression to Alzheimer's disease during the first 12 months of the study (P=0.04), a finding supported by the secondary outcome measures. Among carriers of one or more apolipoprotein E ϵ4 alleles, the benefit of donepezil was evident throughout the three-year follow-up. There were no significant differences in the rate of progression to Alzheimer's disease between the vitamin E and placebo groups at any point, either among all patients or among apolipoprotein E ϵ4 carriers.

CONCLUSIONS

Vitamin E had no benefit in patients with mild cognitive impairment. Although donepezil therapy was associated with a lower rate of progression to Alzheimer's disease during the first 12 months of treatment, the rate of progression to Alzheimer's disease after three years was not lower among patients treated with donepezil than among those given placebo.

From the Mayo Clinic College of Medicine, Rochester, Minn. (R.C.P.); University of California, San Diego, San Diego (R.G.T., D.G., S.J., L.J.T.); Elan Pharmaceuticals, San Diego (M.G.); Rush University Medical School, Chicago (D.B.); Baylor College of Medicine, Houston (R.D.); New York University, New York (S.F.); Oregon Health and Science University, Portland (J.K.); Emory University, Atlanta (A.L.); University of South Florida, Tampa (E.P.); Mt. Sinai School of Medicine, New York (M.S.); and Yale University, New Haven, Conn. (C.H.D.). Address reprint requests to Dr. Petersen at the Alzheimer's Disease Research Center, Mayo Clinic College of Medicine, 200 First St. SW, Rochester, MN 55905, or at peter8@mayo.edu.

*Members of the Alzheimer's Disease Cooperative Study (ADCS) Group are listed in the Appendix.

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Mild cognitive impairment represents a transitional state between the cognitive changes of normal aging and the earliest clinical features of Alzheimer's disease.[1] Amnestic mild cognitive impairment refers to the subtype that has a primary memory component, either alone (single domain) or in conjunction with other cognitive-domain impairments (multiple domain), but of insufficient severity to constitute dementia.[2-6] Previous research has shown that the rate of progression to clinically diagnosable Alzheimer's disease is 10 to 15 percent per year among persons who meet the criteria for the amnestic form of mild cognitive impairment, in contrast to a rate of 1 to 2 percent per year among normal elderly persons.[7] Approximately 80 percent of those who meet the criteria for amnestic mild cognitive impairment will have Alzheimer's disease within six years, and the presence of one or more apolipoprotein (*APOE*) $\epsilon 4$ alleles is associated with a more rapid rate of progression.[8,9] Thus, preventing the progression of mild cognitive impairment to Alzheimer's disease is likely to provide substantial benefit.

Oxidative damage accompanies Alzheimer's disease, and cholinesterase inhibitors are recommended for use in mild-to-moderate Alzheimer's disease.[10] The Alzheimer's Disease Cooperative Study (ADCS)[11] showed that treatment with the antioxidant vitamin E could delay the time to important milestones in patients with moderately severe Alzheimer's disease. The present study was designed to determine whether treatment with vitamin E or donepezil, the most widely used cholinesterase inhibitor available at the time the study was designed, could delay the clinical diagnosis of Alzheimer's disease in subjects with the amnestic form of mild cognitive impairment.

METHODS

PARTICIPANTS

We enrolled 769 subjects from 69 ADCS sites in the United States and Canada.[12] The criteria for inclusion were amnestic mild cognitive impairment of a degenerative nature (insidious onset and gradual progression),[7] impaired memory, a Logical Memory delayed-recall score approximately 1.5 to 2 SD below an education-adjusted norm, a Clinical Dementia Rating (CDR) of 0.5, a score of 24 to 30 on the Mini–Mental State Examination (MMSE), and an age of 55 to 90 years. Detailed inclusion and exclusion criteria are presented in the Supplementary Appendix, available with the full text of this article at www.nejm.org.

STUDY DESIGN

In this multicenter, randomized, double-blind, placebo-controlled, parallel-group study, which was conducted between March 1999 and January 2004, subjects with amnestic mild cognitive impairment were randomly assigned to receive 2000 IU of vitamin E, placebo donepezil, and a multivitamin daily; 10 mg of donepezil, placebo vitamin E, and a multivitamin daily; or placebo vitamin E, placebo donepezil, and a multivitamin daily. The multivitamin contained 15 IU of vitamin E. The initial dose of donepezil was 5 mg daily, and the dose was increased to 10 mg after six weeks. The initial dose of vitamin E was 1000 IU daily, and the dose was increased to 2000 IU (1000 IU twice daily) after six weeks. If a subject had difficulty tolerating the higher dose of vitamin E or donepezil, the investigator could reduce the dose of either medication temporarily and then rechallenge with the higher dose.

We used an adaptive allocation scheme for the treatment assignment, with the MMSE score, age, and *APOE* $\epsilon 4$ status as balancing covariates. The study was designed by the mild-cognitive-impairment protocol committee of the ADCS and was executed and analyzed by the ADCS investigators. Fifty percent of the funding was provided by the National Institute on Aging, with the other 50 percent coming from Pfizer and Eisai. Pfizer and Eisai served in an advisory capacity for the study, but final decisions concerning all phases of the study were made by the ADCS investigators. The study was conducted according to Good Clinical Practice guidelines, the Declaration of Helsinki, and the U.S. Code of Federal Regulations title 21 Part 50 (Protection of Human Subjects) and title 21 Part 56 (Institutional Review Boards). Written informed consent was obtained from all participants and study partners who had knowledge of the participants' functional activities. A data and safety monitoring board reviewed the blinded safety data every three months during the trial.

EFFICACY MEASURES

The primary end point was the time to the development of possible or probable Alzheimer's disease, defined according to the clinical criteria of the National Institute of Neurological and Communicative Diseases and Stroke and the Alzheimer's

Disease and Related Disorders Association.[13] On verification by a central review committee that a participant met these clinical criteria for Alzheimer's disease, the participant stopped taking donepezil or matching placebo in a blinded fashion and was offered open-label donepezil until he or she completed the study at month 36.

Secondary measures were also assessed, including the scores for the MMSE; the Alzheimer's Disease Assessment Scale, cognitive subscale (ADAS-Cog); the global CDR; the CDR sum of boxes (the sum of individual CDR domain scores); the ADCS Mild Cognitive Impairment Activities of Daily Living Scale; the Global Deterioration Scale; and a neuropsychological battery consisting of the New York University paragraph-recall test, the Symbol Digit Modalities Test, the category-fluency test, a number-cancellation test, the Boston Naming Test, the digits-backward test, the clock-drawing test, and a maze-tracing task.[12,14]

STATISTICAL ANALYSIS

The primary analysis was conducted according to the intention-to-treat principle in order to determine whether there was a significant reduction in the time to progression to Alzheimer's disease among subjects treated with either vitamin E or donepezil as compared with those given placebo. The Cox proportional-hazards model was used, and baseline variables (age, the MMSE score, and the *APOE* genotype) were included in the analysis as covariates. Two primary analyses were conducted, one comparing the vitamin E and placebo groups, and one comparing the donepezil and placebo groups. The Hochberg method[15] was used to adjust the two P values for multiple comparisons. The Schoenfeld residuals test was used to test for nonproportional hazards.[16] A z-test (the difference in the proportions divided by the standard error of the difference) was used to compare estimated survival rates at various points on the Kaplan–Meier curves (at 6, 12, 18, 24, 30, and 36 months). The Hochberg method was used to adjust the six P values for multiple comparisons.

Hazard ratios derived from the Cox analysis were used to compare the risk of progression in the donepezil or vitamin E group with that in the placebo group for the entire cohort and for the subgroup of *APOE* $\epsilon 4$ carriers. In the 12- and 24-month analyses, data were censored at 388 and 749 days, respectively. The hazard-ratio analyses were secondary, and the resulting P values were not adjusted for multiple comparisons. Baseline characteristics among the three groups were compared with the use of Wilcoxon's rank-sum test or Fisher's exact test, as appropriate. For the statistical evaluation of main effects, a P value of less than 0.05 was considered to indicate statistical significance, and for interaction effects, a P value of less than 0.10 was used.

The secondary outcomes were examined with the use of analysis of covariance for the change in scores without correction for multiple comparisons, and missing values were imputed with the use of a projection method appropriate for assessing responses among subjects with neurodegenerative diseases.[17] As part of the secondary analyses, several cognitive-domain scores for memory (consisting of the ADAS immediate and delayed word-recall scores and the New York University immediate and delayed paragraph-recall scores), executive function (the digits-backward test, Symbol Digit Modalities Test, and number-cancellation test), language (the Boston Naming Test and category-fluency test), and visuospatial skills (the clock-drawing test) were calculated in addition to an overall composite cognitive-function score. The cognitive-domain and overall composite scores were calculated as the weighted sum of the individual standardized test scores. The individual test scores were standardized by dividing each score by the standard deviation of the baseline scores. Weights were calculated as the reciprocal of the sum of the correlation coefficients between the tests in each domain at baseline.

The annual rates of progression to dementia were calculated with the use of a life-table analysis. An analysis based on a logistic-regression model was conducted to determine whether missing data from subjects who were lost to follow-up were missing completely at random[18] and, if so, could be ignored.

RESULTS

STUDY POPULATION

A total of 790 subjects underwent randomization, and 769 completed the baseline assessment. There were no significant differences among the three groups in baseline demographic or psychometric characteristics (Table 1).

PRIMARY OUTCOME MEASURES

A total of 214 participants had progression to dementia, with 212 being classified as having possi-

Table 1. **Baseline Characteristics of the Subjects.***

Variable	Placebo Group (N=259)	Donepezil Group (N=253)	Vitamin E Group (N=257)	All Subjects (N=769)
Age — yr	72.9±7.6	73.1±7.1	72.8±7.3	72.9±7.3
Female sex — no. (%)	121 (47)	112 (44)	119 (46)	352 (46)
APOE ε4 carrier — no. (%)	136 (53)	147 (58)	141 (55)	424 (55)
ADAS-Cog score				
Original	11.03±4.2	11.28±4.5	11.48±4.4	11.26±4.4
Modified	17.40±6.0	17.72±6.2	18.04±6.0	17.72±6.1
MMSE score	27.35±1.8	27.25±1.8	27.20±1.9	27.27±1.8
CDR sum-of-boxes score	1.87±0.8	1.80±0.8	1.78±0.8	1.82±0.8
Score on Global Deterioration Scale	2.72±0.6	2.66±0.6	2.64±0.6	2.67±0.6
Score on Activities of Daily Living Scale	45.87±5.2	46.49±4.3	45.82±4.6	46.06±4.7

* Plus–minus values are means ±SD. A total of 2264 subjects were screened. The primary reason for exclusion was failure to meet cutoff scores for the Logical Memory paragraph. Scores for the original cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog) can range from 0 to 70, and scores for the modified ADAS-Cog can range from 0 to 85, with higher scores indicating poorer function. Scores for the Mini–Mental State Examination (MMSE) can range from 0 to 30, with higher scores indicating better function. Scores for the Clinical Dementia Rating (CDR) sum of boxes can range from 0 to 18, with lower scores indicating better performance. Scores for the Global Deterioration Scale can range from 1 to 7, with higher scores indicating poorer function. Scores for the Activities of Daily Living Scale can range from 0 to 53, with higher scores indicating better function.

ble or probable Alzheimer's disease, 1 as having mixed dementia, and 1 as having primary progressive aphasia. The overall rate of progression to Alzheimer's disease was 16 percent per year.

During the three years of the trial, there were no significant differences in the probability of progression from mild cognitive impairment to Alzheimer's disease on the basis of the Cox analysis between the vitamin E group and the placebo group (hazard ratio, 1.02; 95 percent confidence interval, 0.74 to 1.41; P=0.91) or the donepezil group and the placebo group (hazard ratio, 0.80; 95 percent confidence interval, 0.57 to 1.13; P=0.42) (Fig. 1A). The Schoenfeld residuals test of nonproportional hazards was significant (P=0.001 for the comparison of the donepezil group with the placebo group and P=0.01 for the comparison of the vitamin E group with the placebo group), indicating that the proportional-hazards assumption for the Cox model was not met. The 36-month analysis was therefore followed by a prespecified assessment of the treatment effects at each six-month evaluation point. This analysis showed that there were no significant differences between the vitamin E and placebo groups at any time during the trial.

The risk of progression to Alzheimer's disease was lower in the donepezil group than in the placebo group for the first 12 months of the trial (P=0.004 at 6 months and P=0.04 at 12 months by a z-test adjusted for comparisons at multiple points) (Fig. 1B). A total of 38 subjects in the placebo group had progression to Alzheimer's disease in the first 12 months, as compared with 33 in the vitamin E group and 16 in the donepezil group. By 36 months, however, the numbers of subjects with progression to Alzheimer's disease did not differ significantly among the three groups: 73 in the placebo group, 76 in the vitamin E group, and 63 in the donepezil group. For the comparison that included all subjects, the hazard ratios for progression to Alzheimer's disease were lower in the donepezil group than the placebo group during year 1 (P=0.004) and during years 1 and 2 (P=0.03), but not during the entire three years of the study (P=0.21; P values not adjusted for comparisons at multiple points).

SECONDARY OUTCOME MEASURES

There were few significant differences in cognitive function from baseline between the vitamin E and placebo groups. The exceptions were in the scores for the executive, language, and overall cognitive scores, and these were confined to the first 18 months of the study. There were more differences in the change from baseline scores between the donepezil group and the placebo group, as shown in Table 2; they included the scores for the MMSE, CDR sum of boxes, Global Deterioration Scale, and modified ADAS-Cog, as well as memory, language, and overall cognitive scores, but with one exception, they were also confined to the first 18 months of the study.

APOE ε4 CARRIERS

Possession of the *APOE* ε4 allele was a major predictor of progression to Alzheimer's disease in all three groups, with 76 percent of the cases of progression to Alzheimer's disease occurring among *APOE* ε4 carriers (P<0.001) (Fig. 1C). There were 136 carriers in the placebo group, 147 in the donepezil group, and 141 in the vitamin E group (Table 1). The curves for the vitamin E and placebo groups separated slightly during the first year, then merged again (P=0.77) (Fig. 1D). In this secondary analysis, it was observed that the curves for the donepezil and placebo groups had separated by six

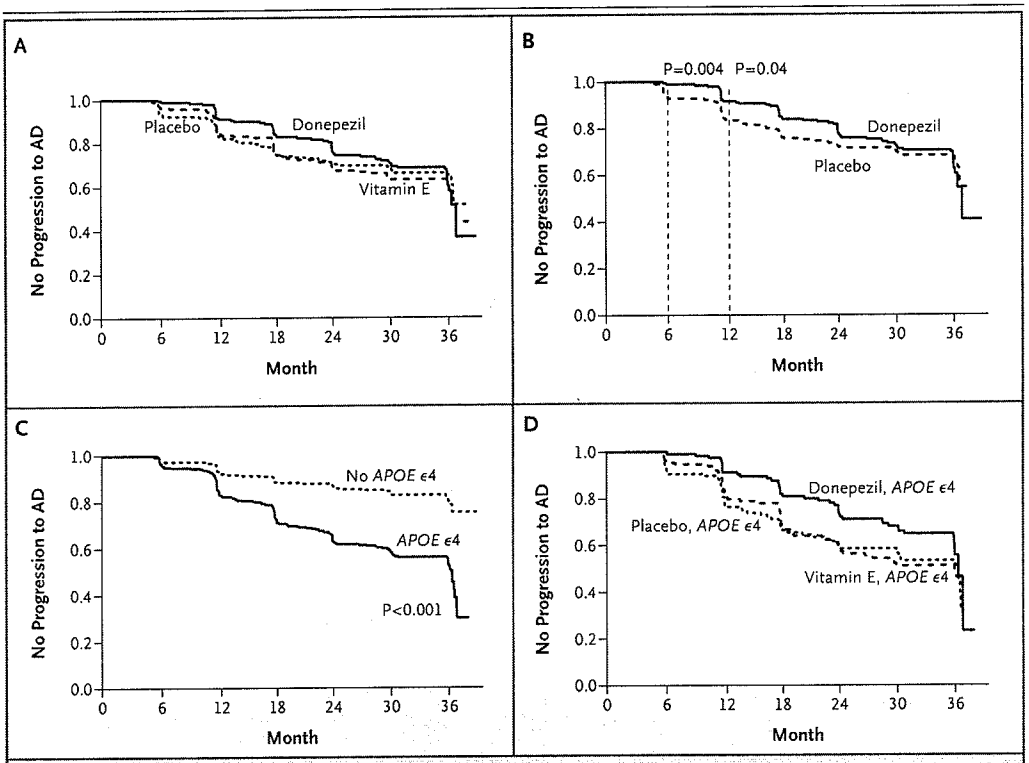

Figure 1. Kaplan–Meier Estimates of the Rate of Progression from Mild Cognitive Impairment to Alzheimer's Disease (AD).
Panel A shows the survival estimates in all three groups during the three-year study. Panel B shows the results of prespecified comparisons involving z-tests at 6 months (P=0.004) and 12 months (P=0.04). Panel C shows the effect of *APOE* ε4 carrier status on the rate of progression to Alzheimer's disease, and Panel D shows the effect of treatment among *APOE* ε4 carriers. Comparisons were adjusted for multiple comparisons with the use of the Hochberg method.

months and remained apart during the remainder of the trial (P=0.04), with donepezil treatment reducing the risk of progression to Alzheimer's disease by approximately one third at year 3 among subjects with one or more *APOE* ε4 alleles (Table 3).

OUTCOMES AND ADVERSE EVENTS

Adverse events in the donepezil group included muscle cramps, gastrointestinal symptoms, and sleep disturbances (Table 4). Twenty-three deaths occurred during the study (17 during the double-blind phase and 6 during the open-label phase), and all were judged to be unrelated to treatment. During the double-blind phase, seven subjects died in the donepezil group and five subjects died in each of the other two groups (P=0.79).

A total of 230 subjects discontinued treatment during the double-blind phase: 92 in the donepezil group, 72 in the vitamin E group, and 66 in the placebo group (P=0.90). Among the leading reasons for discontinuation besides death were adverse events in the case of 47 subjects and withdrawal of consent in the case of 105 subjects.

EFFECT OF MISSING DATA

To assess the effect of missing data, we compared the baseline values between the 230 subjects who withdrew during the double-blind phase and the 539 subjects who progressed to open-label treatment or completed the double-blind phase. There were no significant differences in demographic characteristics or neuropsychological measures. A contingency-table analysis of the number of subjects according to the treatment group and period of withdrawal indicated a trend toward more early dropouts (at the three- and six-month visits) in the donepezil group than in the placebo group (P=0.07). The results of an evaluation of the assumption that the missing data were missing com-

Table 2. **Changes from Baseline in Cognitive and Functional Measures.***

Test	Change in Score from Baseline					
	6 mo	12 mo	18 mo	24 mo	30 mo	36 mo
Cognitive and functional measures						
MMSE						
Donepezil	0.06±2.03†	−0.31±2.25‡	−0.52±2.46‡	−0.98±2.54‡	−1.47±3.04	−2.31±3.72
Vitamin E	−0.53±2.28	−0.54±2.28	−0.96±2.61	−1.21±2.78	−1.75±3.09	−2.20±3.64
Placebo	−0.36±2.02	−0.80±2.34	−1.02±2.61	−1.49±2.90	−1.77±3.24	−2.75±4.04
Activities of Daily Living Scale						
Donepezil	−0.21±3.43	−1.41±4.48	−1.78±5.02	−3.09±6.24	−4.44±7.39	−6.26±8.67
Vitamin E	−0.34±4.29	−1.08±4.90	−2.13±5.76	−2.84±6.16	−4.16±7.46	−5.63±8.75
Placebo	−1.06±4.54	−1.44±5.00	−2.34±6.02	−3.43±6.73	−5.00±8.05	−6.39±8.99
CDR sum of boxes						
Donepezil	0.05±0.66	0.25±0.92‡	0.51±1.18‡	0.87±1.55	1.19±1.69	1.60±2.09
Vitamin E	0.17±0.70	0.51±1.21	0.75±1.44	1.02±1.76	1.26±1.89	1.67±2.18
Placebo	0.14±0.86	0.40±1.28	0.72±1.55	0.97±1.76	1.26±2.15	1.64±2.55
Global Deterioration Scale						
Donepezil	−0.01±0.52†	0.11±0.57	0.19±0.66‡	0.32±0.73	0.45±0.78	0.59±0.89
Vitamin E	0.11±0.49	0.21±0.61	0.27±0.73	0.42±0.80	0.51±0.85	0.64±0.96
Placebo	0.07±0.53	0.15±0.65	0.27±0.73	0.38±0.81	0.48±0.87	0.56±0.99
ADAS-Cog (original)						
Donepezil	−0.61±3.79	0.17±3.73	1.08±4.37	1.22±4.79	2.71±5.21	3.68±5.95
Vitamin E	−0.16±4.19	0.91±4.21	1.19±4.32	1.93±5.13	3.01±5.57	4.59±6.54
Placebo	−0.13±3.34	0.61±4.10	1.29±4.71	1.49±5.07	2.98±5.62	3.74±6.97
ADAS-Cog (modified)						
Donepezil	−1.23±4.74†	−0.55±5.20‡	0.03±5.64‡	0.35±6.23	2.05±6.74	3.12±7.39
Vitamin E	−0.47±5.06	0.27±5.20	0.49±5.42	1.15±6.37	2.48±6.68	3.98±7.56
Placebo	−0.09±4.38	0.60±4.96	0.99±6.07	1.02±6.27	2.65±7.02	3.72±8.54

pletely at random demonstrated that cognitive scores for the MMSE and the ADAS-Cog and total score for the CDR sum of boxes at each visit were predictive of withdrawal before the next visit, indicating that the missing observations cannot be ignored. To assess the z-test results, we conducted a sensitivity analysis consisting of simulations in which the subjects in the donepezil group who dropped out in the first 12 months were randomly divided into two groups: a group of 40 to match the number of dropouts in the placebo group during this period and a group of 24 excess dropouts. A proportion of the 24 excess dropouts was then selected at random and assumed to have had progression to Alzheimer's disease. That proportion was set at the conservative level of double the rate in the group of subjects who completed the study. This analysis included six excess progression events. In these analyses, the 6- and 12-month z-test results remained significant in favor of the donepezil group over the placebo group. The results at all other times were nonsignificant. Similar analyses were performed for the vitamin E and placebo groups, and the results were uniformly nonsignificant.

DISCUSSION

Over the three years of the study, there were no significant differences in the probability of progression to Alzheimer's disease between either the vitamin E or the donepezil group and the placebo group. However, since the effect of treatments varied during the three years of the trial and assumptions for the primary-analysis model were not met, prespecified group comparisons were carried out at each of the six-month evaluations. These analy-

Table 2. (Continued.)*

Test	Change in Score from Baseline					
	6 mo	12 mo	18 mo	24 mo	30 mo	36 mo
Cognitive domains§						
Memory						
Donepezil	0.01±0.51†	0.00±0.57	−0.03±0.57‡	−0.07±0.59	−0.32±0.60	−0.26±0.60
Vitamin E	−0.10±0.48	−0.08±0.49	−0.12±0.55	−0.12±0.57	−0.43±0.55	−0.31±0.59
Placebo	−0.17±0.47	−0.10±0.51	−0.15±0.52	−0.11±0.55	−0.34±0.55	−0.28±0.62
Executive						
Donepezil	0.09±0.36	0.11±0.40	0.03±0.42	−0.01±0.45	−0.06±0.46	−0.16±0.48
Vitamin E	0.11±0.41‡	0.04±0.41	0.00±0.42	0.03±0.45	0.00±0.47	−0.19±0.48
Placebo	0.04±0.42	0.05±0.44	−0.02±0.45	0.01±0.48	−0.08±0.51	−0.19±0.53
Language						
Donepezil	0.09±0.24†	0.04±0.22‡	0.04±0.24†	−0.03±0.25	−0.06±0.29	−0.11±0.32
Vitamin E	0.07±0.23‡	0.05±0.26‡	0.02±0.28‡	−0.03±0.31	−0.05±0.33	−0.10±0.35
Placebo	0.03±0.23	0.00±0.24	−0.03±0.24	0.00±0.27	−0.04±028	−0.08±0.33
Visuospatial						
Donepezil	0.00±0.32	0.00±0.32	−0.05±0.32	−0.06±0.35	−0.14±0.35	−0.14±0.34
Vitamin E	0.03±0.34	−0.01±0.35	−0.02±0.33	−0.04±0.34	−0.07±0.36	−0.12±0.37
Placebo	−0.01±0.34	0.02±0.32	−0.04±0.36	−0.06±0.39	−0.09±0.39	−0.11±0.39
Overall						
Donepezil	0.18±0.82†	0.15±0.92‡	0.01±0.96†	−0.16±1.03	−0.59±1.18	−0.67±1.24
Vitamin E	0.10±0.81†	0.00±0.90	−0.13±0.94	−0.16±1.07	−0.54±1.14	−0.70±1.21
Placebo	−0.12±0.80	−0.03±0.86	−0.24±0.96	−0.15±1.09	−0.53±1.17	−0.65±1.35

* Scores for the Mini–Mental State Examination (MMSE) can range from 0 to 30, with higher scores indicating better function. Scores for the Activities of Daily Living Scale can range from 0 to 53, with higher scores indicating better function. Scores for the Clinical Dementia Rating (CDR) sum of boxes can range from 0 to 18, with lower scores indicating better performance. Scores for the Global Deterioration Scale can range from 1 to 7, with higher scores indicating poorer function. Scores for the original cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog) can range from 0 to 70, and scores for the modified ADAS-Cog can range from 0 to 85, with higher scores indicating poorer function.

† $P<0.01$ for the comparison with the baseline value.

‡ $P<0.05$ for the comparison with the baseline value.

§ The values for the cognitive-domain measures (memory, executive, language, and visuospatial) are standardized composite z scores, with positive numbers indicating improvement. The overall cognitive score was based on the four domain scores and computed as explained in the Methods section.

ses demonstrated that vitamin E had no significant effect during the trial with respect to the development of Alzheimer's disease at any time. The analysis for donepezil, however, demonstrated a reduced likelihood of progression to Alzheimer's disease in the donepezil group, as compared with the placebo group, for the first 12 months of the trial.

These results suggest that donepezil treatment may delay clinical progression to Alzheimer's disease but do not address the question of the underlying mechanism. As shown in Table 2, the overall cognitive function of the subjects with mild cognitive impairment in the donepezil group did not decline on most of the measures during the first 6 to 18 months of the study and thereafter declined at about the same rate as in the placebo group. As a result, the size of the donepezil–placebo treatment effect on the MMSE score was about 0.5 point throughout the 36-month trial. This delay in cognitive decline probably contributed to the slower rate of progression to Alzheimer's disease in the donepezil group. The observed relative reduction in the risk of progression to Alzheimer's disease of 58 percent at one year and 36 percent at two years in the entire cohort is likely to be clinically significant. Although our findings do not provide support for a clear recommendation for the use of donepezil in persons with mild cognitive impairment, they

Table 3. **Hazard Ratios for the Risk of Progression to Alzheimer's Disease in the Donepezil and Vitamin E Groups as Compared with the Placebo Group.***

Interval	All Subjects		*APOE* ε4 Carriers	
	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
Donepezil vs. placebo				
First 12 mo	0.42 (0.24–0.76)	0.004	0.34 (0.16–0.69)	0.003
First 24 mo	0.64 (0.44–0.95)	0.03	0.54 (0.35–0.86)	0.009
All 36 mo	0.80 (0.57–1.13)	0.21	0.66 (0.44–0.98)	0.04
Vitamin E vs. placebo				
First 12 mo	0.83 (0.52–1.32)	0.43	0.78 (0.46–1.34)	0.37
First 24 mo	0.95 (0.67–1.36)	0.79	0.95 (0.64–1.41)	0.79
All 36 mo	1.02 (0.74–1.41)	0.91	0.95 (0.66–1.36)	0.77

* CI denotes confidence interval. P values were not adjusted for multiple comparisons. In the donepezil group, when corrected for multiple comparisons, the P value at 24 months for all subjects became nonsignificant (P=0.052), and the P value at 36 months for *APOE* ε4 carriers also became nonsignificant (P=0.078).

could prompt a discussion between the clinician and the patient about this possibility.

We also found that amnestic mild cognitive impairment and the presence of one or more *APOE* ε4 alleles were highly predictive of progression to Alzheimer's disease. Of the 214 diagnoses of dementia, 212 were possible or probable Alzheimer's disease, with 76 percent of the cases of progression to Alzheimer's disease occurring among *APOE* ε4 carriers. The results show that the enrollment criteria for amnestic mild cognitive impairment were highly specific. Furthermore, this study replicated observational studies demonstrating a rate of progression from mild cognitive impairment to Alzheimer's disease of 10 to 15 percent per year.[5,7]

Treatment with vitamin E and donepezil did not produce any unexpected side effects. No episodes of bleeding occurred in the vitamin E group. There were more discontinuations in the donepezil group than in the other two groups, as would be expected from its known side-effect profile.[10,19] Most discontinuations were related to gastrointestinal side effects, sleep disturbances, and muscle cramps. There were slightly more deaths in the donepezil group, but the number was not out of proportion to the number expected among subjects in this age group and was not significantly different from the numbers in the vitamin E and placebo groups.

We used numerous secondary measures, and in general, they appeared to corroborate the overall outcome data concerning the rate and risk of progression from mild cognitive impairment to Alzheimer's disease. Results for language and the overall composite measure showed some effect of vitamin E therapy, but they were of insufficient magnitude to affect the overall performance of the group. In the donepezil group, the results for memory, language, the overall composite measure, and global measures of cognition, disease severity, and stage of dementia paralleled the overall treatment effect of the drug on the risk of progression to Alzheimer's disease.

Table 4. **Adverse Events.***

Adverse Event	Donepezil Group	Vitamin E Group	Placebo Group
		percent	
Diarrhea	16.7†	10.2	6.6
Muscle cramps	16.3†	1.2	1.9
Insomnia	10.8†	3.1	1.9
Nausea	8.4†	1.2	1.9
Abnormal dreams	6.8†	0.4	1.6
Bronchitis	6.4	2.4	3.1
Loose stools	6.0‡	2.7	1.6
Vomiting	6.0‡	2.7	1.9
Arthritis	5.2‡	2.0	1.6
Cataract extraction	4.8	5.9	2.7

* The rates are for adverse events that occurred in at least 5 percent of subjects in the donepezil or vitamin E group and at least two times in the placebo group during the double-blind phase.
† P<0.01 for the comparison with the placebo group.
‡ P<0.05 for the comparison with the placebo group.

A major modifying effect of the comparison of donepezil with placebo was the *APOE* ϵ4 carrier status. Most of the treatment effect of donepezil occurred among the *APOE* ϵ4 carriers. In secondary analyses, we observed that when the analysis was confined to the *APOE* ϵ4 carriers, the effect of donepezil was significant at 12, 24, and 36 months. However, there are insufficient data to warrant recommending APOE genotyping in persons with mild cognitive impairment, and our results cannot be used to make this recommendation, since the study was not statistically powered to determine the effects of treatment in separate groups of APOE ϵ4 carriers and noncarriers.

Despite evidence of oxidative stress in patients with Alzheimer's disease and mild cognitive impairment and observational studies suggesting that supplementation with antioxidant vitamins may decrease the risk of Alzheimer's disease, we did not find that vitamin E significantly affected the risk of progression.[20-22] Furthermore, this therapy had only minimal effects on secondary measures.

In summary, this study provides evidence that treatment may delay the clinical diagnosis of Alzheimer's disease. Specifically, the likelihood of Alzheimer's disease was reduced for only the initial 12 months of the study among patients treated with donepezil, as compared with those who received placebo; however, in secondary analyses, it was observed that the effect was more prominent among *APOE* ϵ4 carriers, with a reduction in risk apparent throughout the 36 months of the study. The results of the secondary analyses of cognitive and global measures supported the primary-outcome results.

Our findings suggest that the design of our study and the enrollment criteria are practical and can be used to demonstrate the effects of a given intervention in subjects with amnestic mild cognitive impairment. Other therapeutic agents under development, particularly those designed to prevent Alzheimer's disease or progression to Alzheimer's disease, may be particularly beneficial in subjects with mild cognitive impairment.

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APPENDIX

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man, J.M. Olichney, S.M. Johnson, C.W. Jenkins; University of California, San Francisco: K. Yaffe, R. Gearhart, V. Smith; University of Kentucky, Lexington: F. Schmitt, J. Cox, S. Anderson, C. Sowards Dearth; University of Miami, Gulf Coast Education and Research: J. Rivero, R. Ownby, J. Williams; University of Michigan, Ann Arbor: N. Foster, J. Lord, N. Johnson; University of Minnesota, Minneapolis: A. Hochhalter; University of Nevada, School of Medicine: C. Bernick, G. Vranesh, D. Munic, P. LeBlanc; University of New Mexico: J. Adair, S. McClelland; University of Pennsylvania: C. Clark, K. Gravanda, V. Cotter, J. Nuñez, E. Ryan-Ripp; University of Pittsburgh: S. DeKosky, L. Smith-Macedonia, T. Baumgartner, A.L. Kane; University of Rochester Medical Center: P. Tariot, B. Goldstein, L. Terwilliger; University of South Florida, Tampa: E. Pfeiffer, B. Luhn, D. Baxter, J. Hunter; University of Southern California: L. Schneider, N. Taggart, K. Stevens-Dagerman; University of Texas Southwestern Medical Center: M. Weiner, K. Martin-Cook, T. Ninman, S. Pierce; University of Washington: E. Peskind, M. Raskind, R. Wood, N. Brown, J. O'Connell, N. Pham; Veterans Affairs Medical Center, Augusta: M.E. Nichols, C. Bailie, D. Hillesland; Vanderbilt University: R. Margolin, D. Kent, L. McFarland; Washington University School of Medicine: J.C. Morris, S. Stiening, A. Dromerick, C. Dyer; Wien Center: R. Duara, P.D. Roberts; Yale University School of Medicine: C. Van Dyck, M. MacAvoy, L. Cretella, T. Rightmer, L. Zeiser.

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Treatment of the mild cognitive impairment (MCI)

Hervé Allain[1†], Danièle Bentué-Ferrer[1*] and Yvette Akwa[2]

[1]*Laboratory of Experimental and Clinical Pharmacology and Centre Mémoire Ressources Recherche de Bretagne, Faculté de Médecine, 2 avenue du Professeur Léon Bernard, Rennes Cedex, France*
[2]*UMR Inserm 788 and University Paris Sud 11, 80 rue du Général Leclerc, Le Kremlin Bicêtre Cedex, France*

According to Evidence-Based-Medicine, any proposal for the rationale treatment of mild cognitive impairment (MCI) must be based on the results of double-blind, randomized clinical trials (RCTs). However, since MCI at the present time does not constitute a homogeneous clinical syndrome, it is still inappropriate to propose a specific drug treatment. Moreover, RCTs assessing the therapeutic value of acetylcholinesterase-inhibitors (AChEIs) are negative either trying to improve symptoms (memory performance) or preventing the conversion from MCI to real Alzheimer's Disease (AD). The same negative results were obtained with drugs targeting some systems considered as the early steps of the pathophysiological cascade leading to dementia: non-steroidal anti-inflammatory compounds (rofecoxib), sex steroid hormones (testosterone, estrogens), or antioxidants (tocopherol). Either MCI is considered as the very early phase of development of AD (and then the treatments will aim at preventively antagonizing the hallmarks of the disease) or MCI is a new entity (and then the drugs will target the associated neurochemical disturbancies such as tau protein or soluble Aβ oligomers); MCI could also be considered as a monosymptomatic syndrome (amnesia) leading to the development of pure pro-mnestic drugs. These three hypotheses will be presented on the basis of the neurobiology and the pharmacology, and examples of potentially active candidates will be discussed. Copyright © 2007 John Wiley & Sons, Ltd.

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INTRODUCTION

A gradual decline in cognitive performance emerges as one element in longitudinal studies carried out on aging populations. Individual differences can be noticed in the course of this decline, which suggests that some subjects begin developing dementia very early on (Wilson *et al.*, 1999). The presence of insidious Alzheimer's disease (AD) in an aging population is corroborated by neuropathological studies that reveal AD years before the presence of clinical symptoms (Morris, 1999; Morris *et al.*, 2001). In fact, AD can develop primarily in non-demented subjects who present a specific deficit in memory performance; the passage of a symptom (objective memory impairment) to AD (disease), called conversion, with an annual rate of 15%, is considered a transition between normal cerebral aging and AD (Petersen *et al.*, 2001a). This state of transition is now commonly called mild cognitive impairment (MCI), a historic concept and preoccupation, which continues to be the object of debate and numerous classifications (Artero *et al.*, 2006; DeCarli, 2003; Gauthier *et al.*, 2006; Portet *et al.*, 2006; Winblad *et al.*, 2004) (Table 1).

The multiplicity of publications on MCI can be attributed to three main factors: (1) the possibility of early diagnosis of AD; (2) the definition of several potentially predictive parameters of conversion such as homocystein (Annerbo *et al.*, 2005), interleukin-6, low-density lipoprotein (LDL), high ratio of LDL/high-density lipoprotein (HDL), or bioimaging markers (for review see Chertkow, 2002); (3) the possibility of either symptomatic treatments (Allain *et al.*, 2007, in press) to restore cognitive performance

* Correspondence to: D. Bentué-Ferrer, Laboratoire de Pharmacologie, Expérimentale et Clinique, Faculté de Médecine, 2 avenue du Professeur, CS 34317, 35043 Rennes Cedex, France. Tel: +33-(0)2-23-23-45-91; Fax: +33-(0)2-23-23-46-05.
E-mail: daniele.bentue-ferrer@univ-rennes1.fr
† This article is dedicated to the memory of Professor Hervé Allain.

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Table 1. Clinical definitions of cognitive syndromes

Terminology	First described by	Diagnostic criteria
Benign Senescent Forgetfulness	Kral (1962)	Memory loss
Age Associated Memory Impairment (AAMI)	Crook *et al.* (1986)	Subjective memory loss + objective signs, compared to younger subjects
Late-Life Forgetfulness	Blackford and La Rue (1989)	Memory decline + deficient in four (or more) cognitive tests
Age Related Cognitive Decline (ARCD)	Levy (1994)	ARCD for any task
Benign Cognitive Impairment	ICD 10, 1993	Difficulty in cognitive tests secondary to an identified pathology
Mild Neurocognitive Impairment	DSM IV, 1994	Decline in: memory, learning, language or executive functions
Cognitive Impairment No Dementia	Graham *et al.* (1997)	Impairment of cognitive functions with no established dementia
MCI	Petersen *et al.* (1999)	Subjective memory loss associated with an objective memory deficit, adjusted for age and educational background, no dementia

or neuroprotective agents (Akwa *et al.*, 2005) to delay or stop conversion.

The objective of this review is to present the clinical trials carried out on MCI as well as to consider perspectives in drug treatment for an ever-evolving concept (Allain *et al.*, 2002; Sramek *et al.*, 2000) while not forgetting to take into consideration that the pharmaco-economic impact of this kind of approach is of the utmost importance, particularly in terms of public health (Wimo and Winblad, 2003). The current uncertainty in diagnosing, and even the expanded concept itself of MCI, should give rise to much prudence and reserve, as proposed by certain authors (Feldman and Jacova, 2005; Gauthier and Touchon, 2005) who go as far as considering MCI as a risk factor of AD, much like age or apolipoprotein E4. Among the subcategories of MCI that are described, the 'amnestic' form is the best known and studied (Table 2) and therefore the main focus of pharmacological studies (Jelic and Winblad, 2003; Jelic *et al.*, 2003).

METHODS AND PITFALLS

1) The collected elements were obtained by searches of the most popular data bases—Medline, PubMed, Pascal—by combining the following: MCI, Memory disorders, Drugs, Pharmacology, Neurochemistry, Treatment, Alzheimer disease. The search covered the period 1960–2006. Reviews and original articles related to the topic were retained. Additional papers of interest were chosen from the reference lists of the identified articles.
2) The obvious risks in undertaking such a review on MCI treatment deserve to be presented again (Budson and Price, 2005; Petersen *et al.*, 2001b; Rivas-Vazquez *et al.*, 2004): (1) a lack of a single accepted definition of MCI and low-to-moderate accuracy of MCI criteria used in drug trials (Visser *et al.*, 2005); (2) a plethora of neurochemical hypotheses and hence potential pharmacological targets, but a lack of solid correlations for MCI mechanisms and diagnosis; (3) uncertainty about prognostic criteria; (4) contrast between the rarity of clinical studies on MCI and the large number of studies on older concepts (e.g., Age Associated Memory Impairment [AAMI], Age Related Cognitive Decline [ARCD], Benign Cognitive Impairment); (5) usual rarity of publications with negative results.
3) According to the principles of Evidence-Based-Medicine, the therapeutic possibilities for MCI should be based on conclusive clinical trials (mainly Phase III) of high methodological quality. The therapeutic strategies to be explored should stem from neurobiological studies, particularly those devoted to memory (Lynch, 2002; Miyaschita, 2004). Drugs or active agents primarily studied are now chosen based on epidemiological or physiopathological data relative to AD and analyzed at early or predementia stages (Andreasen and Blennow, 2005; Chong and Sahadevan, 2005; Doraiswamy, 2003; Ihl, 2003).

Table 2. Operational criteria of amnestic MCI

1. Memory loss, if possible, corroborated by a third party
2. Objective deficit in memory performance adjusted for age and education level
3. Global cognitive functions retained
4. Integrity of everyday activities
5. Absence of severe dementia

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4) Developing drugs for the treatment of MCI should follow the classic course from hypothesis, then Phase I in humans, and obtaining the definitive proof in Phase III. The objective of such an approach is to either symptomatically improve cognition, and more specifically, memory performance (pro-mnestic drugs), or antagonize MCI progression (physiopathogenic approach). Phase II clinical trials, using healthy volunteers or patients, play a pivotal role as they demonstrate drug action, the reality of the mechanistic hypothesis, the active posology, and initial safety elements. These studies (bridging-studies, proof-of-concept-studies), which are reserved to a few specialized investigation centers, rely on clinical, biological, and neuroimaging evaluations, carried out on limited patient samples, and, logically, condition the course of further clinical development. This calls for a close collaboration between neurobiologists, psychologists, neuropsychologists, and pharmacologists as part of academic and industrial teams, for reasons of cost and methodological quality (Allain *et al.*, 2002; Chong and Sahadevan, 2005).

MAJOR CLINICAL TRIALS

In 2006, few major clinical trials on MCI treatment were published. Most available results are either negative or inconclusive. The content of the trials found in the literature is instructive and should ideally lead to revising the methodology of these clinical trials and also to better define biological drug targets. Main characteristics of randomized, double-blind, placebo-controlled trials in MCI are presented Table 3.

Acetylcholinesterase-inhibitors (AChEIs)

- There are numerous underlying neurobiological factors in the choice of AChEIs in MCI treatment: (1) Phase III randomized clinical trials (RCTs) are in favor of a slowing-down of the progression of AD (Coyle and Kershaw, 2001); (2) the potentially neuroprotective effect is described (role of muscarinic receptors in neurotrophic regeneration; restoration of nicotine receptor activity) (Francis *et al.*, 2005; Geerts, 2005); (3) inhibition of β-amyloid plaque formation by impacting secretion of amyloid precursor protein (APP) is proved (Riepe, 2005).
- The three AChEIs currently on the market were evaluated for MCI using similar methodologies and protocols. Rivastigmine (Exelon®) was tested in a large trial on 1000 patients aimed at delaying conversion (Geula *et al.*, 2000). Johnson and Johnson Pharmaceutical Research and Development made public the negative results of combined studies which included 2048 aged people who received 8 or 12 mg galantamine (Reminyl®) or placebo twice daily for 24 months (Mayor, 2005). Neither of the two MCI trials (GAL-INT-11, DeKosky, and GAL-INT-18, Winblad, unpublished data) found significant treatment effect in term of ADAS-cog at 12 or 24 months. DeKosky's trial reached marginal significance in terms of dementia conversion (www.clinicalstudyresults.org). Koontz and Baskys (2005), in a double-blind placebo-controlled study, including 35 patients, found an improvement of working memory performance and global functioning in patients with MCI with galantamine. According to the Cochrane review by Loy and Schneider (2006), the use of galantamine in MCI is not recommended due to its association with an excess death rate. Donepezil (Aricept®) was first evaluated in a multicenter study on 270 patients labeled as having amnestic MCI over a period of 24 weeks and was found ineffective based on a delayed recall memory test as the main evaluation criterion (Salloway *et al.*, 2004). An attempt to explain this negative result that can be generalized to other AChEIs, clearly poses the question of the basis for using this drug class in MCI treatment, for instance the unrationale attitude to stimulate the observed upregulation of the cholinergic tone accompanying MCI (Allain *et al.*, 2004). More recently, Petersen *et al.* (2005) published the results of a large scale study comparing donepezil, vitamin E, and placebo on the progression of amnestic MCI to established AD in 769 subjects over a period of 3 years. Results show that the rate of progression to AD after 3 years was not lower in the donepezil group than in the placebo group (16% per year). However, donepezil did lower the rate of conversion by a third in subjects with one or more *APOE ε4* alleles, an observation that raises the future possibility of more individualized MCI treatment according to identified risk factors. Birks and Flicker (2006), in their Cochrane Review, concluded that there is no evidence to support the use of donepezil for patients with MCI. The putative benefits are minor, short lived, and associated with significant side effects.

Other treatments

Antioxidants. Trials are underway, as yet unpublished, aiming at prove that antioxidants (vitamin E, selegiline, *Ginkgo Biloba*) slow or stop MCI to AD

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Table 3. Main characteristics of randomized, double-blind, placebo-controlled, parallel-group trials in MCI

Compounds, dose	Number of subjects and age	Inclusion criteria	Duration	Primary outcome	Conclusions	Sponsor, references
Acetylcholinesterase-inhibitors						
Donepezil, 10 mg daily	769 (placebo 259, donepezil 253, Vitamin E 257) 55–90 years	MMSE 24–30, CDR 0.5 Degenerative, amnestic MCI	3 years	Progression to AD	Lower rate of progression during the first 12 months but not after 3 years. No benefit with vitamin E	NIA, Pfizer, Eisai Petersen *et al.* (2005)
Donepezil, 5 mg then 10 mg daily	270 (donepezil 133, placebo 137), 55–90 years	MMSE $\geq$24, CDR 0.5 ADL $\leq$1.5 HAM-D $\leq$12	24 weeks	NYU Paragraph Delayed Recall, ADCS CGIC-MCI	No effect on primary efficacy measures	Pfizer, Salloway *et al.* (2004)
Galantamine, 16–24 mg daily	995, >50 years	Amnestic MCI, NYU Paragraph Delayed Recall $\leq$10, CDR 0.5	24 months	Progression to AD	No effect on conversion rate	Johnson & Johnson GAL-INT-11
Galantamine, 16–24 mg daily	1062, >50 years	Amnestic MCI, NYU Paragraph Delayed Recall $\leq$10, CDR 0.5	24 months	Progression to AD	No effect on conversion rate	Johnson & Johnson GAL-INT-18
Galantamine, 8–12 mg twice daily	19 (galantamine 8, placebo 11), 51–87 years	MMSE $\geq$26, Peterson criteria	16 weeks	CANTAB	Significant improvement on two of the six subtests	Janssen, Koontz and Baskys (2005)
Rivastigmine, 3–12 mg daily	1018, mean 70.5 years	Amnestic MCI, NYU Paragraph Delayed Recall <9 CDR 0.5, HAM-D $\leq$13	Up to 4 years	Progression to AD	Negative	Novartis, InDDex
Dopamine receptor agonists						
Piribedil, 50 mg daily	60 (piribedil 30, placebo 30), > 60 years	MMSE 21–25	90 days	MMSE score	Significant increase (> 26) in MMSE with piribedil	Nagaraja and Jayashree (2001)
Sex steroid hormones						
Testosterone enanthate, 100 mg IM weekly	32 (17 MCI, 10 testosterone, 7 placebo), 63–85 years	Amnestic MCI, MDRS	6 weeks	Neuropsychological tests	Improvements in spatial memory and constructional abilities	Cherrier *et al.* (2005)
Anti-inflammatory agents						
Rofecoxib, 25 mg daily	1457 (rofecoxib 725, placebo 732), $\geq$65 years	Amnestic MCI MMSE $\geq$24 CDR = 0.5 BDRS $\leq$ 3.5 HAM-D $\leq$ 13	Up to 4 years	Progression to AD	Diagnosis of AD not delayed	Merck, Thal *et al.* (2005)

ADCS CGIC-MCI, Alzheimer Disease Cooperative Study Clinician's Global Impression of Change for MCI; ADL, Activities of Daily Living; BDRS, Blessed Dementia Rating Scale; CANTAB, Cambridge Automated Neuropsychiatric Test Assessment Battery; CDR, Clinical Dementia Rating; MMSE, Mini Mental State Examination; HAM-D, Hamilton Rating Scale for Depression; IM, intramuscular; MDRS, Mattis Dementia Rating Scale; NYU, New York University.

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conversion (Brenner, 2003; Mecocci *et al.*, 2004) but the trial of Petersen *et al.* (2005) failed to demonstrate a benefit from vitamin E in MCI. This approach is in line with previous studies on preceding concepts on MCI with cognitive stimulants and nootropics (Levy, 1994; Lockhart and Lestage, 2003). Results are consistently disappointing and contrast with neurobiological data that have always been in favor of the role of free radicals and Radical Oxygen Species (ROS) in cell death and dementia onset (Qin *et al.*, 2006).

Dopamine receptor agonists. Cerebral aging is accompanied by a progressive reduction in central dopamine receptors, correlated with a decrease in cognitive performances (Volkow *et al.*, 2000). In parallel, dopamine agonists have been shown to mostly have an antioxidant effect. The randomized controlled-study by Nagaraja and Jayashree (2001) reported a significant improvement in Mini Mental State Examination (MMSE) scores in the group treated with the D_2/D_3 dopamine receptor agonist piribedil, versus the placebo arm, in 60 patients with suspected MCI.

Sex steroid hormones. In a bridging study, Cherrier *et al.* (2005) studied the effect of weekly injections of 100 mg of testosterone enanthate in 15 AD and 17 MCI patients versus placebo over 6 weeks. The supplementation with testosterone appeared to be beneficial in the two groups of patients, particularly in terms of spatial memory and praxies. This is in line with theoretical reports on the beneficial role of androgens in treating andropause (Tan *et al.*, 2003).

Supplementation with estrogens was based on observational studies as a reasonable treatment for MCI. However, recent RCTs, testing conjugated equine estrogens alone or associated with progestins do not support the use of such hormones for treating MCI, preventing AD or cognitive decline (Mulnard *et al.*, 2004). This observation is corroborated by the large scale randomized, double-blind, placebo-controlled clinical trials of the Women's Health Initiative Memory Study, which also demonstrated no effect and even increase in risk of dementia or cognitive decline in post-menopausal women (65 years and older) supplemented with conjugated equine estrogen alone (0.625 mg/day) or in combination with medroxyprogesterone acetate (2.5 mg/day) for 7–9 years (Craig *et al.*, 2005; Shumaker *et al.*, 2004).

Anti-inflammatory agents. A randomized, double-blind, placebo-controlled study tested the hypothesis of the efficacy of a COX-2 selective inhibitor, refecoxib (25 mg/day), on MCI to AD conversion. This large scale trial (725 patients in the active group vs. 732 in the placebo group), carried out over a 4-year period, could not demonstrate refecoxib's superiority to placebo. Facing those results, some authors abandoned exploring the idea of inflammation not only in MCI but also in AD (Thal *et al.*, 2005).

DISCUSSION

Initial attempts to obtain proof of efficacy of drugs for treating MCI (both symptoms and conversion) have failed, and there is currently no proven therapy for MCI. This is a harsh reality that calls into question current hypotheses as it widens the oft-exposed gap between neuroscientific data and clinical observations, again recently raised with memantine, an uncompetitive NMDA-R antagonist prescribed in late stages of AD (Volbracht *et al.*, 2006). The impression of regular failure is in sharp contrast with the plethora of publications on the subject (Thal, 2003) and the wide variety of drugs tested in humans, often sporadically or without perseveration (e.g. see Table 4).

For the future, clinical and neurochemical objectives need to be more clearly defined. Research for a drug that acts purely on symptoms and memory performance calls for precise targeting of the biological bases of memory and, in bridging studies, centering studies around the analysis of the different specific components of human memory through adapted psychometric batteries (Allain *et al.*, 2007, in press). According to this hypothesis, the primary criterion in Phase III trials should focus on memory performance. Inversely, drugs that aim to antagonize a physiopathogenic or a proven etiopathogenic process should remain centered on the advanced hypothesis. For instance, the highly suspected neurotoxicity of the β-amyloid (Aβ) protein in MCI has lead to anti-amyloid pharmacological approaches (Table 5) (Bentué-Ferrer and Allain, 2006). The list of potential anti-amyloid therapies increases daily and can be presented according to the targeted steps in the Aβ peptide synthesis or elimination processes: (1) decrease in formation of the neurotoxic Aβ peptides (secretases, cholesterol); (2) acceleration of Aβ peptide clearance (active or passive immunization); (3) antagonizing of protein aggregation (glycosaminoglycanes inhibitors, chelators). Although numerous possibilities exist, the main difficulty, particularly at

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Table 4. Main drugs studied to treat symptoms or prevent conversion from MCI to AD

Acetylcholinesterase-inhibitors	Donepezil, galantamine, rivastigmine	Koontz and Baskys (2005); Salloway *et al.* (2004); Petersen *et al.* (2005);
AMPAkines	CX516	Johnson and Simmon (2002)
Anti-amyloids	β-Secretase and γ-secretase inhibitors; glycosaminoglycan [GAG] inhibitors; immunotherapy	Bentué-Ferrer and Allain (2006), for review
Anti-inflammatory agents	Anti-Cox 2	Thal *et al.* (2005)
Antioxidants	Vitamin E; selegiline; acetyl-L-carnitine	Brenner (2003); Montgomery *et al.* (2003); Morris *et al.* (2002); Petersen *et al.* (2005)
Dopaminergic receptor agonists	Piribedil	Nagaraja and Jayashree (2001)
Sex steroid hormones	Estrogens, testosterone	Cherrier *et al.* (2005); Mulnard *et al.* (2004); Tan *et al.* (2003)
Lipid lowering agents	Statins	Sparks *et al.* (2005)
Membrane modulators	Citicoline or CDP-choline	Abad-Santos *et al.* (2002)
Nootropics	Piracetam	Waegemans *et al.* (2002)
PPAR-gamma agonists	Rosiglitazone	Watson *et al.* (2005)

the industrial level, is to gather the best results obtained *in vitro* and in animal models to begin the first studies in humans. Certain drugs look promising like Alzhemed (Geerts, 2004) or serotonergic 5-HT_3 receptors antagonists (Ban and Seong, 2005). These molecules have been well evaluated in animal models (Akwa *et al.*, 2005) and respond to the description of cerebral anomalies observed in certain stages of MCI or early AD, published by Morris and Price (2001). As far as future pro-mnestics are concerned, care should

Table 5. Anti-amyloid pharmacological strategies

Strategy	Target	Pharmacological approach
Diminish the formation of neurotoxic Aβ peptides	α-secretases	Protein kinase C activators; Muscarinic M_1 and M_3 receptor agonists; Metabotropic glutamate receptor agonists; Serotonergic 5-HT_{2A} and 5-HT_{2C} receptor agonists
	β-Secretases	Inhibitors: OM99-1; OM99-2; OM00-3; OM99-2 analogues; GT-1026
	γ-secretases	Inhibitors: Peptide mimetic aldehyde (calpain inhibitors); Peptide mimetic difluoroketone; Difluoroketone analogues; L-685,458; LY-45139 (Phase II); Fenchylamine sulfonamides DAPT (dipeptide) R-flurbiprofen (Phase III)
	Cholesterol	Lipid lowering agents; Statins
Favor Aβ peptide clearance	Immunological compounds	Active immunization: AN-1792 vaccine (trial interrupted at Phase IIa); Passive immunization: antibodies to Aβ, AAB-001, humanized monoclonal antibody (Phase II)
Interfere with the Aβ aggregation process and plaque formation	Metal ions	Chelators: clioquinol
	Aggregation inhibitors	Rifampicin, tetracyclines, apomorphine, cyclohexanehexol, nicotine, melatonin, inositol, β-cylodextrins, Congo red, 2,4 dinitrophenol, pyridone derivatives
	Glycosaminoglycanes (GAG)	Inhibitors: Alzhemed, Cerebril, Fibrillex
	β-sheet conformation	Inhibitors: peptides ($iA\beta_{11}$, $iA\beta_5$)
Act on immediate consequences after onset	Neuroinflammation	Anti-cyclooxygenase-2

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be taken, as new neurobiological results have changed our understanding of memory mechanisms and disorders and hence drug targets (Budson and Price, 2005). For example, the study conducted by Gais and Born (2004) confirmed that in young, healthy volunteers, low cholinergic tone is necessary during sleep for memory consolidation. This could explain the absence of effect of AChEIs on memory and MCI, knowing that AChEIs are usually given to obtain a pharmacokinetic steady-state without respecting the necessary decrease in night central cholinergic tone.

The definition of new targets for future compounds is dominated by the debate about the neurotoxicity of proteins such as tau or Aβ, and the possibility of pharmacologically antagonizing an either direct or indirect toxic mechanism (Rao *et al.*, 2006) on the basis of animal and *in vitro* models. A recent example concerns the potential link between memory decline, neurofibrillary degeneration (NFD), and tau protein (SantaCruz *et al.*, 2005). In transgenic mice suffering from NFD, neuronal loss, and behavior disturbances, suppression of tau protein expression restored memory and stabilized the number of neurons, though NFD accumulated. This suggests that a promnesiant drug, in the case of tauopathy, should antagonize the tau protein. Doglio *et al.* (2006) demonstrated that inhibiting tau phosphorylation in a transgenic fly model expressing human tau, which causes neurotoxicity in the fly eye, could be a safe approach to reducing neurotoxicity. As far as Aβ peptide is concerned and according to Lesné *et al.* (2006), the extracellular accumulation of a specific soluble dodecameric (56 kDa) Aβ assembly, in the brain of middle-aged transgenic mice *Tg257*, causes memory deficits before any onset of detectible histological lesions. This suggests that any drug acting upon peptide aggregation may be considered as a potential candidate to prevent AD (Maudsley and Mattson, 2006). In that respect, it has recently been shown that a new candidate, cyclohexanehexol stereoisomer, inhibits aggregation of Aβ into high-molecular weight oligomers in the brain and ameliorates several AD like phenotypes in an AD transgenic mouse model (McLaurin *et al.*, 2006).

Overall, pharmacology is rife with drugs that could either improve memory and certain components of cognition or that oppose the physiopathological processes leading to AD. Pharmacologists and neurobiologists await certitudes before testing in Phase III drugs (and hypotheses) in MCI. The recent discovery in AD transgenic mouse models that a soluble Aβ oligomer impairs memory before any onset of objective lesions, deserves to be researched as a potential biomarker in humans with amnestic MCI before undertaking any drug trials. In addition, concentrations of total tau, phosphorylated tau 181, and $A\beta_{1\text{-}42}$ in cerebrospinal fluid are strongly associated with future development of AD in patients with MCI (Hansson *et al.*, 2006). It is important to note that the present approach for MCI could logically be extended to other neurodegenerative disorders and primarily Parkinson's disease (Fernandez *et al.*, 2005; Park *et al.*, 2006) to be treated at their earliest stages.

Sadly, Herve Allain died before this paper was published. As a memorial note these few words can hardly do justice to such a willing and constant supporter, both as editor and contributor, of Human Psychopharmacology; Clinical & Experimental. Herve was always enthusiastic about each and all of his broad range of research interests within neurology, clinical psychopharmacology, bioethics and methods and measures in clinical trials. His greatest enthusiasm was, however, reserved for his students, both undergraduate and postgraduate, and his devotion to teaching and training was reciprocated in the enduring respect and affection of those who had the pleasure of benefiting from his typically exciting and erudite didactics. Herve Allain had seemingly endless energy for teaching and research: not simply the delivering of countless lectures and the publishing of a myriad of first-rate papers, monographs, reports and books, but also the time consuming activity associated with the spirited membership of numerous committees, professional bodies, regulatory agencies, advisory panels and conference organisations. Herve's contribution to the neurobiology and psychopharmacology of memory and his work on Alzheimer's disease will remind many of our loss. Herve once joked that the advantage of a surname like Allain was that you were always placed near the top of lists when something was to be distributed. For me, Herve Allain was a good friend, and at the top of most lists of distinction within neuro-psychopharmacology, not by alphabetical quirk but by right and justly so.

Ian Hindmarch

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